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THE AMERICAN JOURNAL OF PATHOLOGY

VOLUME XX MARCH, 1944 NUMBER 2

CONCRETIONS IN THE ANTERIOR PITUITARY LOBE OF THE HUMAN EMBRYO AND THE NEWBORN *

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While examining pituitary glands of newborn children, the senior author, years ago, was struck by the finding of colloid † concretions in the anterior lobe. A search of the literature concerning them yielded little information. Erdheim mentioned the finding of a few calcific concretions in the anterior lobe of a man, 56 years old, who died of carcinoma of the esophagus. He distinguished them from the meningeal concretions as found on the capsule of the pituitary gland.

Lucien and Parisot saw small, round, calcific concretions in the anterior lobe in one case; they did not state age or sex.

Kraus, in a paper on the lipoid substances in the hypophysis, casually mentioned that he had seen, repeatedly, concentric laminated calcific concretions in the anterior lobes of embryos and young infants. In later writings, Kraus referred to the same finding as obviously nonpathologic.

Most of the numerous textbooks and monographs that were consulted do not refer to the subject. The occasional mention of concretions, *i.e.*, von Gierke in Aschoff's textbook, 1936, leaves one in doubt whether the colloid concretions within the anterior lobe are referred to or the calcific bodies in the meningeal investment of the organ. Rasmussen's paper deals only with the latter. An extensive, but necessarily incomplete, survey of pertinent embryologic, experimental and endocrinologic literature was fruitless.

This paper will not deal with pituitary concretions as found in various diseases (for instance, Simmonds' disease, adiposogenital dystrophy, hemorrhage in anterior lobe, tumors of anterior lobe). We are concerned only with concretions originating in the normal anterior lobe and the pars intermedia, especially in the fetus and the newborn.

* Received for publication, May 1, 1943.

† Throughout this paper the word "colloid" is used in a general sense, without commitment as to chemical nature, and in default of any other fully acceptable term.

Controls

Failure to observe concretions in a fairly extensive experience with the adult human pituitary gland could not be accepted as a satisfactory negative control. Therefore, 110 pituitary glands from persons more than 1½ years of age (most of them between 40 and 70 years) were subjected to special search. Of these, 6 showed numerous concretions comparable to the ones in the newborn pituitary body; the ages were 3, 46, 52, 56, 65 and 68 years. In 8 instances, only one, or very few, concretions were found. In this group the ages were 8, 13, 45, 51, 53, 57, 60 and 62 years. In each of these 8 cases 50 sections were carefully searched. In 6 of the negative controls, more than 60 sections were examined; in 11, more than 50; in 20, more than 40. In 8 of the negative controls, sections were systematically studied under high magnification. Neither age distribution nor sex distribution of the positive controls was significant. No disease or group of diseases was prevalent among the positive controls. In 5 instances, with only few sections available, no decision could be reached as to the presence or absence of concretions.

Material and Methods

The pituitary glands of 150 newborn children and young infants were examined. The organs were fixed in formaldehyde, embedded in paraffin and the sections stained with hematoxylin and eosin. Some were studied serially. On the average, about ten sections of every pituitary gland were available. In all newborn infants, including several anencephali, concretions were present. They were found also in most of the infants who had died before the sixth postnatal month. The youngest age at which concretions were absent was 2 months (male, 58 cm. long; female, 50 cm. long). In one male, 48 cm. long, who had lived for 2 months and 7 days, very many concretions were present. Of the three 3-months-old infants the first had very few concretions; the second, none; the third revealed only one doubtful concretion in a series of 192 sections. There was only one specimen from the fourth postnatal month; it contained concretions in very small numbers. There were three from the fifth month: one positive, the second with very few, the third one negative. But all of the four pituitary glands of the sixth postnatal month did contain concretions, one in moderate numbers, one few, two very few. The 7½-months-old infant had few, one from the eighth month none. In the 19 sections of the anterior lobe of a boy, 1½ years old, none could be found; but there were occasional concretions in the pituitary gland of a boy, 2 years and 2 months old, with 49 sections available. Thus, in spite of the small

number of cases, the conclusion seems warranted that the concretions tend to decrease or disappear in early infancy. In the pituitary gland of the embryo and the newborn the concretions were found easily; in all other age groups one had to search for them patiently. There was no difference in the occurrence of concretions between males and females.

In most specimens a magnification of 75 × was sufficient. Some slides were studied at 160 × magnification and in selected cases we made sure of the absence of even the smallest concretions by searching many fields under still higher magnification (280 ×).*

Fourteen embryonic pituitary glands were studied; they all contained concretions, mostly in large or moderate numbers. The two smallest embryos measured 55 mm. crown-rump length; in one, few were found; in the other very many, up to 30 in one section.

Location of Concretions

Practically all of the concretions in the infants and the older fetuses were situated in the anterior lobe proper. Only an exceptional one was seen in the pars intermedia or the pars tuberalis. The smallness of these areas may be a sufficient explanation. The distribution of concretions in the anterior lobe in relation to periphery and center, neighborhood of the cleft or the stalk, did not seem to follow any rule. The majority of the concretions were surrounded by epithelial cells in irregular fashion, without prevalence of one of the three cell types. About one concretion out of ten appeared to be surrounded by epithelial cells with a more or less radial arrangement. This, in accordance with the histologic picture, was more frequent in younger embryos. The lumen, in which such concretions seemed to be situated, might be a shrinking space. The anterior lobe contains so many capillaries that, by necessity, some concretions must be situated near them. Occasionally the picture was that of a concretion in the lumen of a capillary (Fig. 13). Colloid in a sinusoid of the anterior pituitary lobe is not unusual; thus, a colloid concretion might be intracapillary as well. But it is possible also that the concretion has torn the delicate capillary wall while the paraffin block was being cut.

Description of Concretions

More than half of the concretions were ovoid, about one-fifth were irregular in shape, some were round, and a few pyramidal. The average size was 18 by 13 μ, the largest one was 80 μ long; there were many

* Since these investigations were concluded, many more pituitary glands of newborn children and of adults have been examined. Concretions were found regularly in the newborn.

small ones down to 4 μ in diameter. There was no significant variation in size with age and sex. However, in a few younger embryos, large concretions were conspicuous in the anterior lobe and in the pars intermedia. The concretions assumed a great variety of hues in the hematoxylin and eosin stain. Most of them stained purplish blue, some were a pure blue, others pinkish blue; only a few were pink or red. Some, which appeared to be very hard structures, did not become deep blue with the hematoxylin and eosin stain, as calcific masses are supposed to do. The hardness of the concretions was attested to by their breaking and by the frequent tearing of surrounding tissues.

In a human embryo of 55 mm. crown-rump length,* many, partly very large, concretions were found. Some of them, reaching a length of 80 μ, were visible with a hand lens. They were club-shaped, sausage-shaped or irregularly pyramidal. One, which was 70 μ long, had several gentle indentations. Since the average thickness of the large concretions was 10 to 12 μ, some of the small concretions might represent cross sections of large ones. There were, however, numerous very small particles with the same optical characteristics. They were mostly situated in spaces which were surrounded by epithelial cells while the large concretions were found in solid epithelial masses. At the lower surface of the pituitary gland a group of concretions seemed to lie outside the epithelial organ in a kind of "bay" as if the surrounding epithelial cells had retracted from it (Fig. 1). Probably these relatively large, firm masses had led to pressure atrophy of the epithelial cells by which they were formed. Figure 5 from the pars intermedia of an embryo, 9 cm. long, may represent an earlier phase of this process. Only with reluctance can one accept such pictures as denoting a "normal" developmental process.

About half of the concretions were more or less laminated (Figs. 9, 10, 12, 13, 14 and 20). In embryos up to 29 cm. crown-heel length, lamination was seldom distinct. On the whole, indistinct lamination was three times as frequent as distinct lamination. The concretions, laminated or not, revealed their character as solid bodies by their irregular shape and by festooned or jagged edges. Colloid masses, which before fixation were fluid or semifluid, have a smooth, regularly convex contour. The presence, size and shape of shrinking spaces next to the concretions could not be used as an indicator of their consistency.

Large Calcific Concretions

In five cases the anterior lobe contained larger calcific masses which were different from the colloid concretions (female, 57 years, pheno-

* Columbia Study Collection, no. 2034, courtesy of Dr. Tracy J. Putnam.

barbital poisoning; male, 60 years, carcinoma of bladder; male, 40 years, septic endocarditis; male, 50 years, diabetes; female, 37 years, rheumatic heart disease). These concretions were situated in the most dorsal portion of the anterior lobe, an area which often is rather fibrosed in older persons. They reached one-tenth, in one of the cases almost one-third, of a millimeter in diameter (Figs. 7 and 8). They essentially represented thin concentric shells. But there also were compact calcific masses; they stained inky blue with hematoxylin; they did not take any eosin. Some of the thin fragments remained unstained and appeared highly refractive and light brown.* These concretions bear a certain resemblance to those which Wislocki found in the anterior lobe of a 30-year-old elephant. The concretions in the pituitary gland of the elephant definitely were connected with the colloid. Since these larger concretions in the human adult are situated in a region of the anterior lobe which normally is rich in connective tissue, one cannot assume scarring in their neighborhood. But in two of these five cases, indistinct giant cells were found near the calcific masses, indicating foreign body action. As previously mentioned, we have never seen any tissue reaction around the ordinary colloid concretions in the newborn. Possibly these large concretions do represent calcification of large colloid masses which are surrounded by connective tissue directly, the epithelium having disappeared. Inside the calcific ring in Figure 8 the colloid is still recognizable and atrophic epithelium is seen in the neighborhood.

The lancet-shaped calcific mass within colloid, shown in Figure 19, represents an isolated observation.

Concretions in Erdheim Glands

In one instance only were concretions found in Erdheim glands (Figs. 15 and 17). They are different from the other concretions in their aspect and, more important, in their partly intracellular location. One might assume that the cell formed its product but did not expel it. Little seems to be known about the secretory mechanism of the Erdheim glands (Rasmussen). Figure 16 definitely shows secretion granules in the epithelial cells of an Erdheim gland.

Concretions in Other Fetal Organs

The question arises whether or not concretions are found during fetal life in organs other than the pituitary body. No systematic search in

* Obviously Erdheim has seen such concretions in a 20-year-old patient who died of heart disease. Erdheim described them as concentrically laminated lime capsules which formed large agglomerations.

this direction has ever been undertaken as far as we know. But a few observations are available. In the otherwise normal kidney cortex of a full-term male infant, 5 hours old, a laminated concretion was found. It was surrounded by normal tubular epithelium. The pyramids in this kidney did not contain concretions.

In the fat tissue outside the suprarenal gland of a male fetus, 20 cm. long, small, ovoid, structureless, calcific masses were situated.

Hassall's corpuscles sometimes calcify during fetal life, completely or partially. This change has been observed by Hammar and we have repeatedly seen it.

In the epithelial lamella which connects glans and prepuce during fetal life, horny pearl-like bodies are regularly present. Stieve mentioned their calcification in the newborn. We have seen a partly calcified one in a fetus of 22 cm. crown-heel length. Identical structures can be found in the corresponding lamella of the preputium clitoridis.

We have never seen concretions in the thyroid of the fetus or the newborn. In these stages the thyroid follicles contain little or no colloid. But even in the adult thyroid concretions are very seldom encountered.

The cysts, which in the adult so frequently are found between the anterior and posterior lobes of the pituitary gland, often are distended with dense colloid matter but they never contain concretions. Thus, inspissation alone does not explain the forming of the concretions. If, as we may assume, colloid masses in the pituitary gland are similar to those found in the thyroid, their water content is very low, giving small leeway for inspissation.

Comment

Since the concretions are found in the pituitary gland of the fetus and the newborn in practically 100 per cent of the specimens examined, we have to consider these findings statistically normal. The question whether they also conform with an idealistic norm may appear gratuitous; it may seem a relic from times when man believed in the infallibility of creative nature. But the question may help us in a certain way. These concretions are found in the embryo and in the newborn; they tend to disappear in early postnatal life. Hence it seems logical to assume that some maternal influence is responsible for their formation and maintenance. The findings thus can be considered together with other phenomena which are caused by hormonic, or other, maternal influence. Such findings are: colostrum secretion in the newborn, hyperemia and hemorrhage in the endometrium of the newborn, maturing of ovarian follicles in the embryo, enlargement of the uterus, hypersecretion of cervical glands, squamous metaplasia in the prostate.

In 1914, Alfred Kohn, the anatomist in Prague, in a very interesting paper, discussed these and similar findings. He designated the whole complex of these findings in the newborn as a "miniature puberty." * He mentioned that even acne, such a typical phenomenon of puberty, occurs in the fetus and in the newborn. He also pointed out, in this connection, the hypertrophy of the suprarenal gland with the postnatal degenerative processes, and the enormous masses of interstitial cells in the ovary and testicle of the equine fetus. These interstitial cells decrease toward the end of intrauterine life and disappear in the first postnatal month. In the testicle of the human fetus, also, interstitial cells are very numerous. As Kohn puts it, we are dealing here with transitory phases of irritation caused by substances coming from the maternal organism.

The placental circulation exposes the embryo to influences which are not entirely adequate, since substances for which the embryo may have no use are carried to its organs, *e.g.*, the sex hormones, even of the opposite sex. The changes caused by the hormones do not, according to present-day knowledge, damage the embryo. Pressure atrophy of epithelium, however, as caused by the pituitary concretions (Fig. 5), borders on the pathologic.

The distention of hair follicles and of sebaceous gland ducts, as found to a high degree in acne of the newborn, and to a lesser degree in almost every newborn, represents a picture which would be called seborrhea or comedo in the adult. That means it would be considered as a disease.

Finally, erythroblastosis fetalis is a fatal disease in which incompatibility between maternal and fetal blood plays a rôle. Such a disease could hardly be imagined in an oviparous species. To date our knowledge of fetal and neonatal disease is fragmentary. It remains to be seen what rôle similar incompatibilities between mother and fetus play in unexplained death of the fetus, especially in habitual abortion.

Summary

1. Colloid concretions are a constant finding in the anterior pituitary lobe and in the pars intermedia of the human fetus and the newborn.

2. Most of the concretions disappear in the first postnatal months.

3. Large calcific bodies are sometimes found in the anterior pituitary lobe of the adult.

4. We believe that the concretions in the fetus are formed under the influence of maternal hormones, thus adding to the group of conditions

* This expression was first used by Jacquet and Rondeau in 1905. These authors, however, did not believe that the phenomena in question were caused by the hormones of the mother.

in which there are biologic and possible pathologic implications of maternal hormone action upon the fetus.

5. In one instance, concretions which were partly intracellular were found in Erdheim glands.

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DESCRIPTION OF PLATES

PLATE 41

FIG. 1. Embryo no. 2034, Columbia Study Collection, 55 mm. crown-rump length. Concretions next to thinned-out epithelial lining of pituitary cavity. × 360.

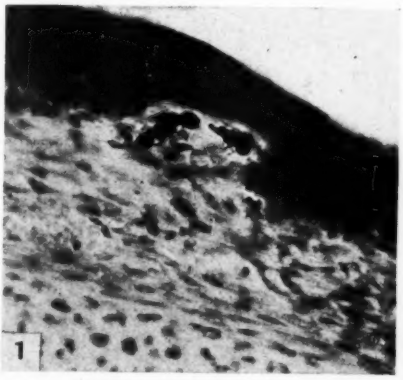
FIG. 2. Same specimen as shown in Figure 1. Large, irregularly shaped concretion in lining of pituitary cavity. × 360.

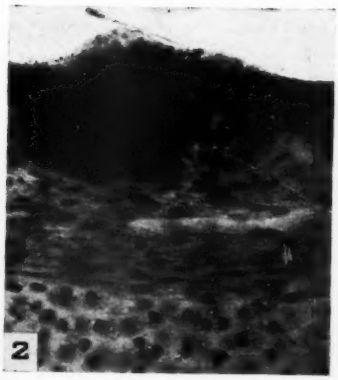
FIG. 3. Same specimen as shown in Figures 1 and 2. General view of pituitary region. Sphenoid cartilage at bottom of photomicrograph; brain ventricle at top. A large concretion is present in the left lower corner. × 80.

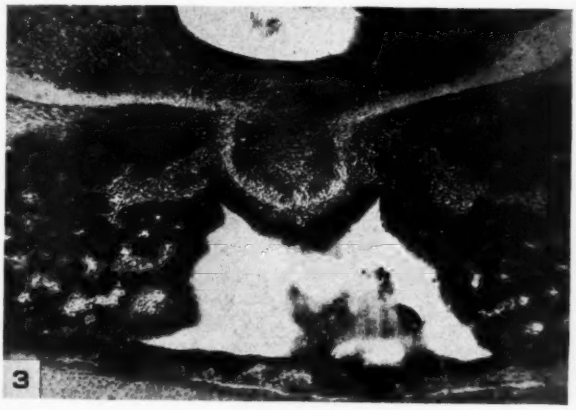
FIG. 4. Same specimen as shown in Figures 1 to 3. Sphenoid cartilage just visible in left lower corner. Anterior lobe tissue with many concretions, four of which are in focus. × 360.

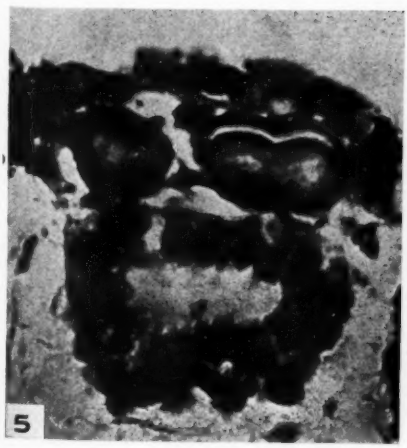
FIG. 5. Path. no. 35948, 9 cm. crown-rump length. Sex unknown. Epithelial lining of pituitary cavity thinned out by concretions. × 750.

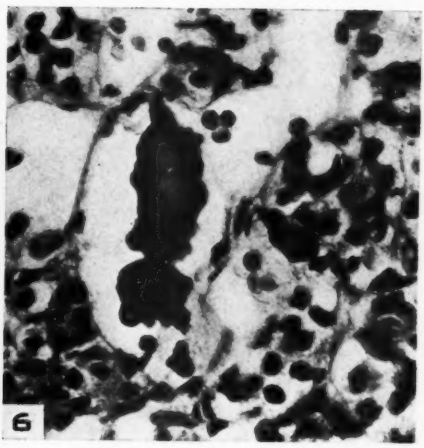
FIG. 6. A-44-40, female newborn, lived for 32 hours. Anatomic diagnosis: multiple hemorrhages. Large, irregularly shaped colloid concretion in anterior lobe, a smaller one next to it. × 670.











Plaut and Galenson Concretions in the Anterior Pituitary Lobe

PLATE 42

FIG. 7. Adult. No clinical data available. Large, broken, calcific masses in anterior lobe. × 600.

FIG. 8. A-49-41, female, 57 years old, who died of phenobarbital poisoning. Calcific ring at periphery of colloid mass. An ordinary colloid mass, still surrounded by flattened epithelial cells, is seen in the right upper corner of the photomicrograph. The field is taken from the somewhat fibrosed posterior portion of the anterior lobe. × 220.

FIG. 9. A-70-33. Male anencephalus, 1 day old. Concretion with much variation in density of different layers. No shrinkage space around concretion. × 700.

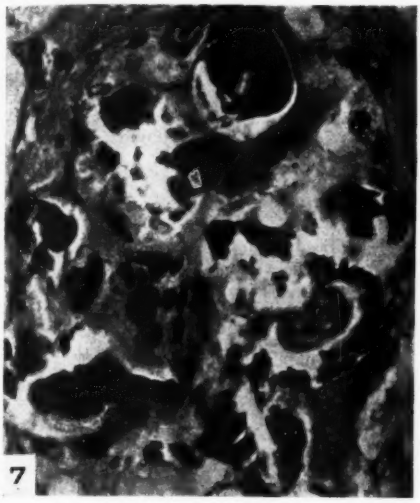
FIG. 10. A-111-38, female, 19 days old. Multiple malformations; mongolism. Small colloid concretion with beaded edge and laminated margin. The center appears homogeneous. × 700.

FIG. 11. A-53-38, female, 1 day old. Intracranial hemorrhage. Typical colloid concretion. Lamination is visible. The edge is partly beaded. The surrounding tissue appears unaltered. × 750.

FIG. 12. A-107-37, male, 10 hours old. Doubtful pneumonia. Dense beaded edge. Distinct lamination. × 900.

FIG. 13. A-186-35, female, 21 days old. Cerebellar hypoplasia with meningocele. Compact concretion, perhaps intracapillary, with three spindle-shaped nuclei surrounding it. × 900.

FIG. 14. A-48-37, male stillborn, 43 cm. crown-heel length. Multiple hemorrhages. Colloid masses with faint traces of lamination. The capillaries are very wide, as frequently found in the newborn. × 900.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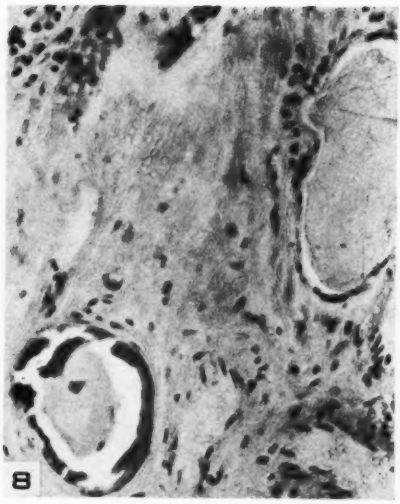
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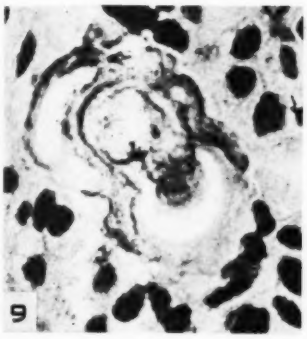
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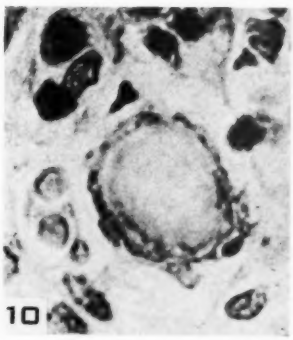
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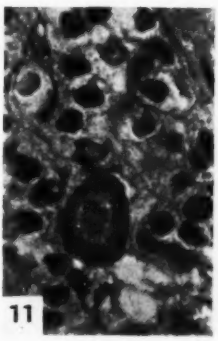
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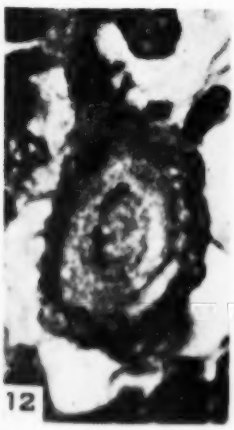
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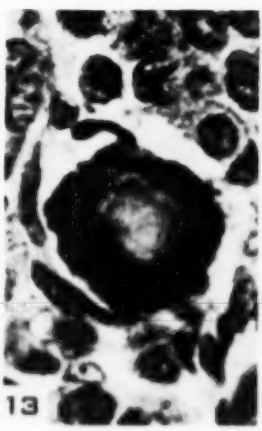
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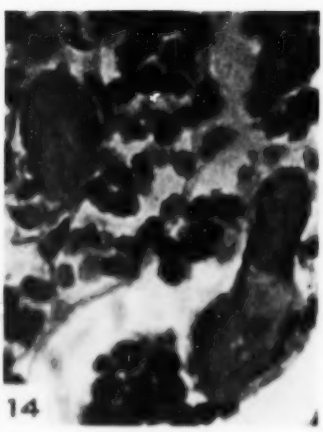
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Plaut and Galewsky — Concretions in the Anterior Pituitary Lobe

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DESCRIPTION OF PLATES

PLATE 41

FIG. 1. Embryo no. 2034, Columbia Study Collection, 55 mm. crown-rump length. Concretions next to thinned-out epithelial lining of pituitary cavity. × 360.

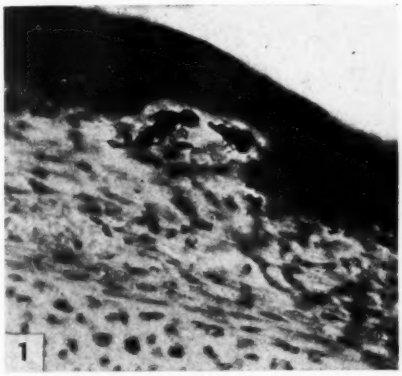
FIG. 2. Same specimen as shown in Figure 1. Large, irregularly shaped concretion in lining of pituitary cavity. × 360.

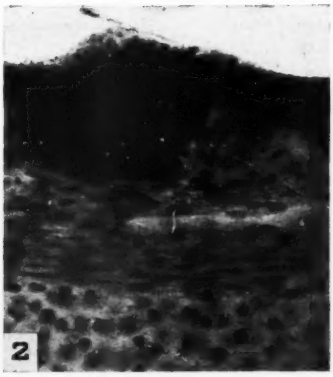
FIG. 3. Same specimen as shown in Figures 1 and 2. General view of pituitary region. Sphenoid cartilage at bottom of photomicrograph; brain ventricle at top. A large concretion is present in the left lower corner. × 80.

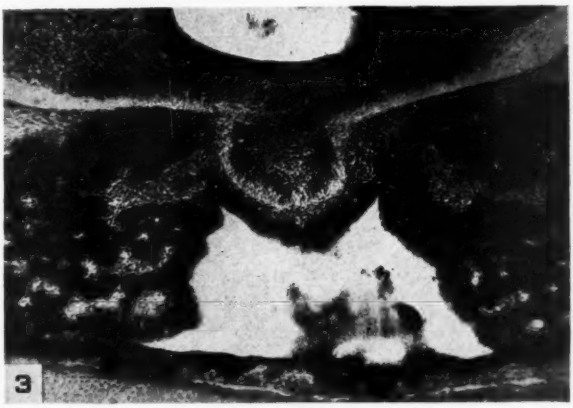
FIG. 4. Same specimen as shown in Figures 1 to 3. Sphenoid cartilage just visible in left lower corner. Anterior lobe tissue with many concretions, four of which are in focus. × 360.

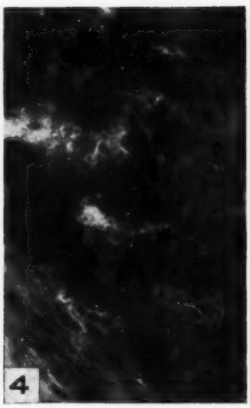
FIG. 5. Path. no. 35948, 9 cm. crown-rump length. Sex unknown. Epithelial lining of pituitary cavity thinned out by concretions. × 750.

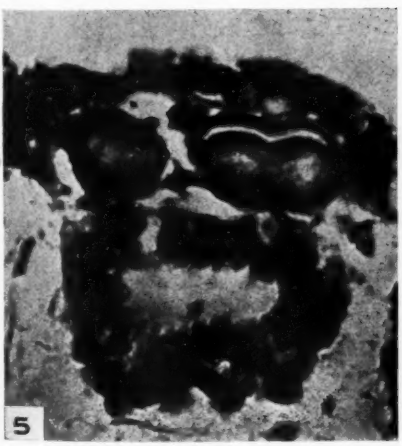
FIG. 6. A-44-40, female newborn, lived for 32 hours. Anatomic diagnosis: multiple hemorrhages. Large, irregularly shaped colloid concretion in anterior lobe, a smaller one next to it. × 670.

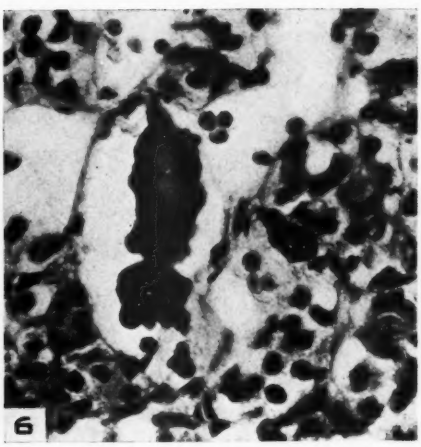












Plaut and Galenson Concretions in the Anterior Pituitary Lobe

PLATE 42

FIG. 7. Adult. No clinical data available. Large, broken, calcific masses in anterior lobe. × 600.

FIG. 8. A-49-41, female, 57 years old, who died of phenobarbital poisoning. Calcific ring at periphery of colloid mass. An ordinary colloid mass, still surrounded by flattened epithelial cells, is seen in the right upper corner of the photomicrograph. The field is taken from the somewhat fibrosed posterior portion of the anterior lobe. × 220.

FIG. 9. A-70-33. Male anencephalus, 1 day old. Concretion with much variation in density of different layers. No shrinkage space around concretion. × 700.

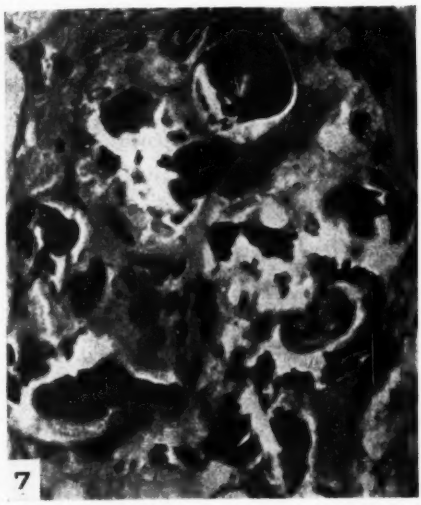
FIG. 10. A-111-38, female, 19 days old. Multiple malformations; mongolism. Small colloid concretion with beaded edge and laminated margin. The center appears homogeneous. × 700.

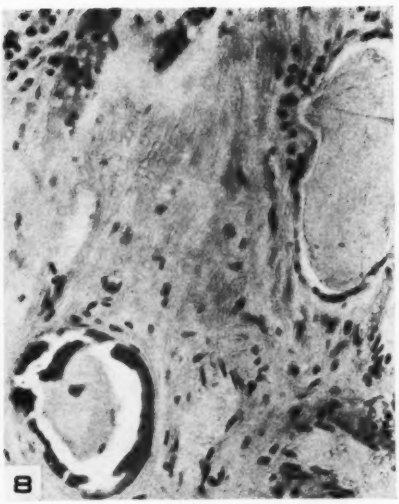
FIG. 11. A-53-38, female, 1 day old. Intracranial hemorrhage. Typical colloid concretion. Lamination is visible. The edge is partly beaded. The surrounding tissue appears unaltered. × 750.

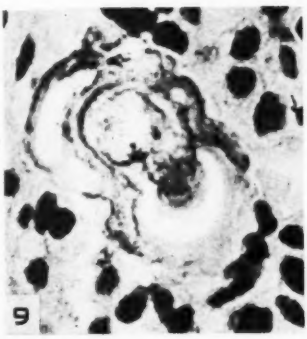
FIG. 12. A-107-37, male, 10 hours old. Doubtful pneumonia. Dense beaded edge. Distinct lamination. × 900.

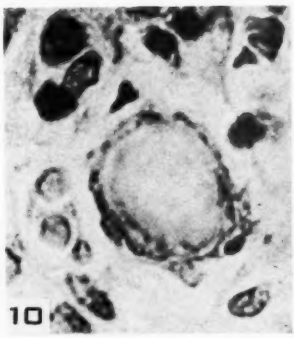
FIG. 13. A-186-35, female, 21 days old. Cerebellar hypoplasia with meningocele. Compact concretion, perhaps intracapillary, with three spindle-shaped nuclei surrounding it. × 900.

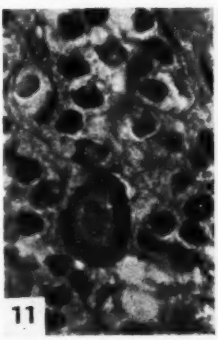
FIG. 14. A-48-37, male stillborn, 43 cm. crown-heel length. Multiple hemorrhages. Colloid masses with faint traces of lamination. The capillaries are very wide, as frequently found in the newborn. × 900.

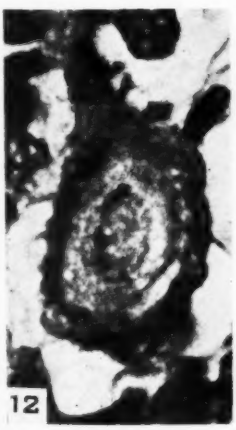


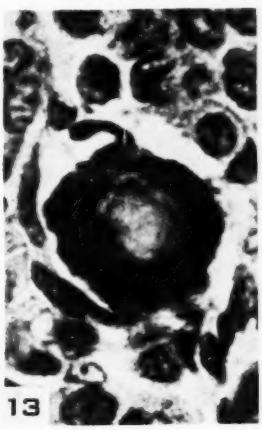


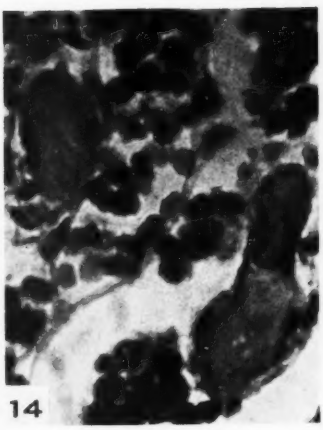












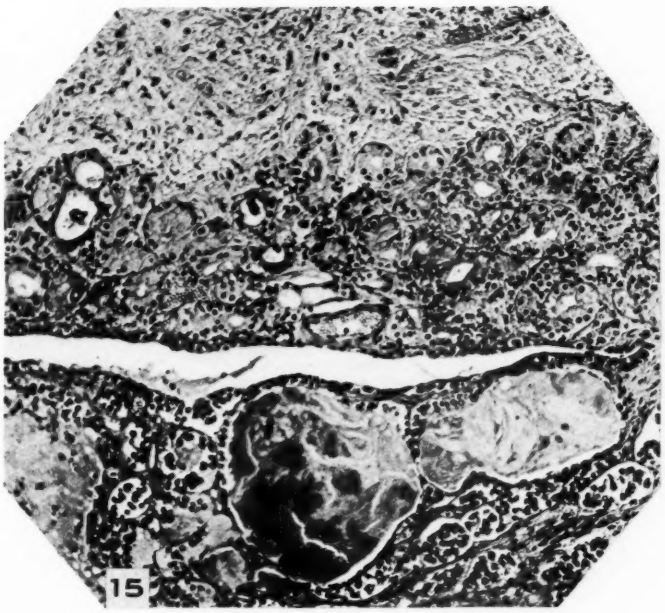
Plaut and Galenson Concretions in the Anterior Pituitary Lobe

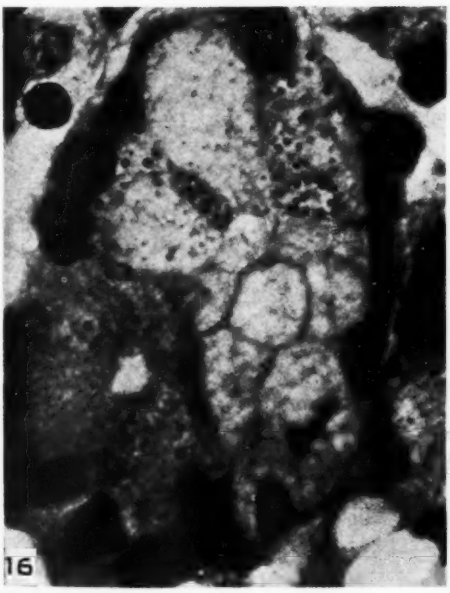
PLATE 43

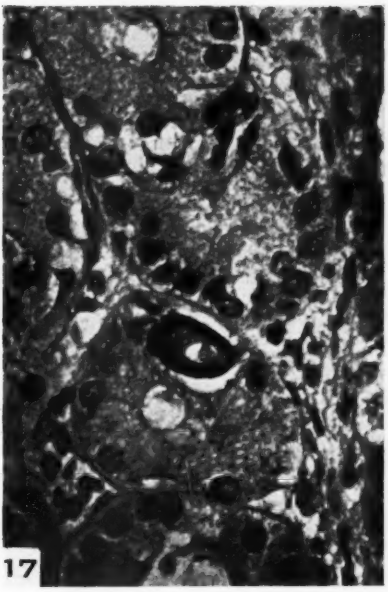
FIG. 15. A-36-34, male 13 years old. Tuberculous meningitis. The hypophyseal cleft runs horizontally through the photomicrograph, separating the anterior lobe (below) from the posterior lobe (above). Erdheim glands occupy an unusually large area next to the cleft. They contain three concretions (left half of picture). The two upper ones are luminal, the lower one is intracellular. × 150.

FIG. 16. A-176-35, female stillborn, 50 cm. crown-heel length. Secretion granules in Erdheim gland. Nuclei at base. × 1500.

FIG. 17. The left lower concretion from Figure 15, at higher magnification. The concretion is situated within an epithelial cell. The narrow round lumen of the Erdheim gland is seen below the concretion. × 670.







Plaut and Galenson

Concretions in the Anterior Pituitary Lobe

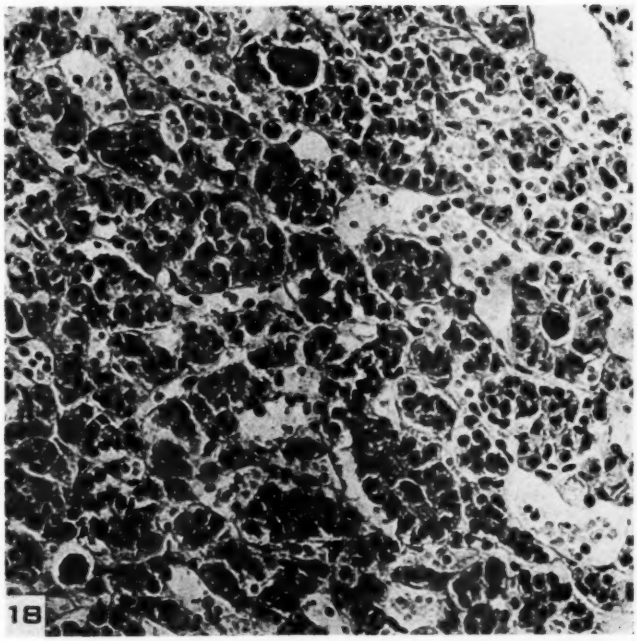
PLATE 44

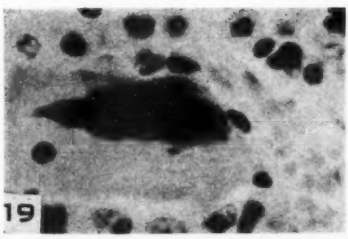
FIG. 18. A-134-39, male stillborn, 58 cm. crown-heel length. Intracranial hemorrhage. Four colloid concretions in one microscopic field. One of them is not distinct. ×250.

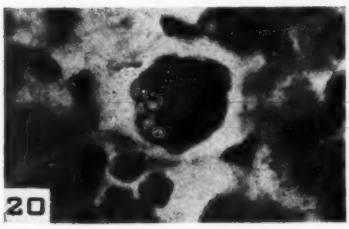
FIG. 19. A-134-32, female, 24 years. Rupture of cerebral aneurysm. Lancet-shaped calcific mass in colloid. × 670.

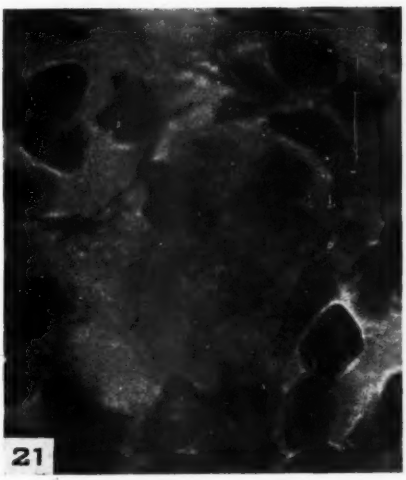
FIG. 20. A-21-35, female, 3 days old, 33 cm. crown-heel length. Congenital syphilis. Very small colloid concretion with distinct lamination and scalloping of edges. This is a type frequently seen. × 960.

FIG. 21. A-13-36, female newborn, 50 cm. long. Subdural hemorrhage, aspiration of vernix. Concretion-like mass of varying density without distinct outline; this, perhaps, is a concretion in disintegration. Only parts of the concretion are in focus. (Photomicrograph taken with pinpoint diaphragm.) × 1500.









Plaut and Galenson Concretions in the Anterior Pituitary Lobe

THE TESTIS IN VITAMIN E-DEFICIENT GUINEA-PIGS *

ALWIN M. PAPPENHEIMER, M.D., and CLAUDIA SCHOGOLEFF
(From the Department of Pathology, College of Physicians and Surgeons, Columbia University, New York, N. Y.)

Loss of fertility associated with testicular degeneration is a familiar and well studied effect of vitamin E deficiency in the white rat. The very careful work of Mason[1-4] and others has made clear the underlying histopathology. The change is irreversible;[5, 6] once effected, the testicular structure and function cannot be restored by vitamin E administration. Daily doses of 0.75 mg. of alpha-tocopherol protect the testis against degeneration.[7]

This striking result of vitamin E deficiency has thus far been demonstrated only in the rat. Bryan and Mason[8] have shown that mice on vitamin E-deficient diets do not develop testicular degeneration even after 400 days, and this is confirmed by our own experience.[9, 10] Mackenzie and McCollum[11] have found that muscular dystrophy may be produced in rabbits in the absence of testicular degeneration. Only 3 of 11 rabbits used in their experiment received no tocopherol supplement. They were maintained for periods of 49 to 79 days on the vitamin E-deficient diet. While these authors do not draw from their experiments the conclusion that vitamin E is unessential for the integrity of the rabbit's testis, their observations do indicate that the skeletal muscle in this species is more sensitive than is the testis to lack of vitamin. Probably because of the difficulty in raising guinea-pigs beyond maturity on simplified vitamin E-deficient diets, there has been no previous study as to the rôle of vitamin E in preserving the integrity of the testis in this species. Since we have succeeded in maintaining guinea-pigs in good condition for several months after sexual maturity on a diet deficient in vitamin E, the opportunity has been offered to study the condition of the testis in such animals.

METHODS

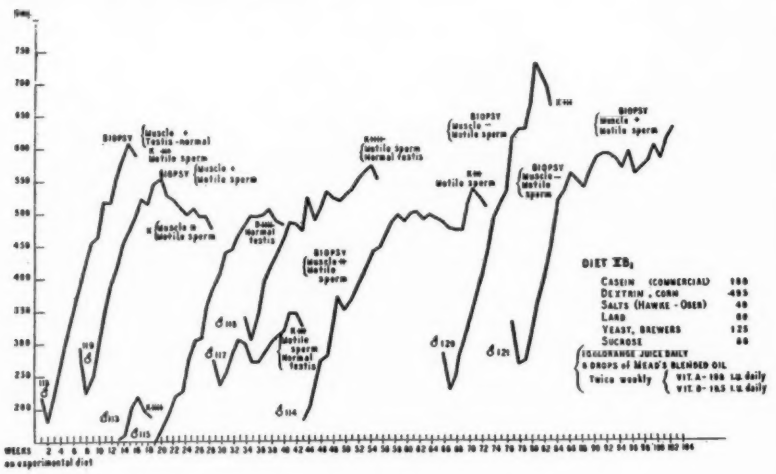
Guinea-pigs were placed on vitamin E-deficient diets soon after weaning. Of 17 animals used in the experiments, 3 received the standard diet V used by Pappenheimer and Goettsch[12] in previous studies on muscular dystrophy, but it was supplemented by aqueous lettuce extract or linseed meal, or both.[13-15] Cod liver oil was incorporated in the diet. In spite of these additions, growth was poor and

* Received for publication, May 17, 1943.

dystrophy developed between the 60th and 85th days. The testes were normal.

Five other guinea-pigs were given a diet in which dextrin was substituted for cornstarch. In some the diet was treated with ethereal ferric chloride before adding dextrin; in others, the complete diet was treated. All developed severe dystrophy, and the survival period did not exceed 70 days on the diet. The testes, in those animals which survived until after maturity, were normal. A series of 9 guinea-pigs was then given diet V B_1. In the hope of prolonging life, fish liver oil was not incorporated into the diet, but given separately twice weekly by pipette. The diet was mixed into a paste with water, dried with an electric fan, broken into small lumps and fed without additional roughage.

On this diet, the onset of muscular dystrophy was delayed, and the growth of 7 of the 9 animals was excellent, averaging 3.2 gm. daily weight gain for the first 70 days. Eventually all developed typical muscular lesions (Text-Fig. 1).



Text-Fig. 1. Guinea-pigs on a vitamin E-deficient diet.

The vitamin E content of diet V B_1 was tested on 3 pregnant rats, which were placed on the diet immediately after mating. All had resorptions.

The guinea-pigs were sacrificed after they had shown evident symptoms of muscle weakness. The motility of the sperm from vas deferens or epididymis was examined at the time of autopsy, and the testis fixed

in Zenker's fluid for histologic study. In several animals, previous specimens of one testis and muscle were obtained for biopsy, and the motility of the sperm tested.

Experimental Findings

Our observations on diet V B_1 are summarized in Table I. Because of their better growth and longer survival period, the animals in this group have yielded the most informative data.

The testes of 3 animals, killed after 105 days on the diet, presented a normal gross and histologic structure. Motile sperm were obtained from all.

After 130 to 139 days on the vitamin E-deficient diet, 2 of 4 animals showed early degenerative changes in some tubules, from swelling and clumping of the spermatozoa to complete disappearance of spermatozoa and spermatids (Fig. 1). The other 2 animals had normal testes. Motile sperm were present in the epididymides of all.

Guinea-pig 114, from which a normal testis was obtained by hemicastration after 131 days, was killed on the 165th day. The testis then contained a moderate number of degenerated tubules, but motile sperm were still present in the epididymis. Guinea pig 121, having a normal testis on the 84th day, had developed a very advanced atrophy of the remaining testis when killed on the 175th day (Fig. 2). The organ was shrunken, flabby and distinctly brown. Spermatogenesis was absent; the tubules were lined with Sertoli cells, and contained occasional larger elements filled with brownish pigment. The appearance corresponded to Mason's [1] stage V in rats. In spite of the extreme degeneration, motile sperm could still be obtained from the epididymis.

Attempts to prove the fertility of these animals by matings with normal females on stock diet have thus far not succeeded. Seven matings after the males had been from 62 to 112 days on the vitamin E-deficient diet have all been sterile.

The skeletal muscles of all guinea-pigs on diet V B_1 have shown characteristic dystrophic lesions (Figs. 3 and 4).

Discussion

These experiments, like those of Mackenzie and McCollum [11] with rabbits, demonstrate that muscular dystrophy in guinea-pigs may be produced while the testis is still histologically normal and elaborating actively motile sperm. They do not bring proof that vitamin E is unessential for spermatogenesis. Testicular degeneration was advanced in the single animal that survived for 175 days, while early degenera-

TABLE I

Muscular Dystrophy and Testicular Findings in Guinea-Pigs on a Vitamin E-Deficient Diet

No.	Days on diet V B_1*	Initial weight	Maximum weight	Final weight	Muscular dystrophy	Mobile sperm	Testicular structure	Incidental lesions
		(gm.)	(gm.)	(gm.)				
117	K105	280	342	322	+++	+105 days	Normal	—
118	K105	216	605	535	+++	+ 96 days +105 days	Normal	—
120	K105	286	730	650	+++	+105 days	Normal	—
115	D130	150	500	365	++++	?	Normal	——
116	K132	340	560	430	++++	+132 days	Swelling and agglutination of sperm (Mason stage I)	Lipoid pneumonia
114	Biopsy 131				++	+131 days	Normal	—
119	K139	296	552	470	++	+139 days	Few tubules degenerated	Hemorrhages over thorax; necrosis of liver
114	K165	180	525	498	+++	+165 days	Moderate number of degenerated tubules	—
121	K175	335	675	635	+	+ 84 days +163 days +175 days	Normal Complete degeneration	—

* Diet VB_1: Commercial casein, 180 gm.; dextrin (corn), Merck N.F.V. 495 gm.; salts (Hawke-Oser), 40 gm.; lard, 80 gm.; brewer's yeast, 125 gm.; sucrose, 80 gm.; 10 cc. of orange juice daily; 8 drops of Mead's blended fish oil twice weekly, stated to be equivalent to 108 I.U. of vitamin A and 10.5 I.U. of vitamin D.

tive changes in some tubules were observed in guinea-pigs sacrificed at periods from 130 to 165 days.

In the rat, degeneration of the testis is usually present after 40 to 50 days on a vitamin E-deficient diet begun immediately after weaning. From experiments reported in the following article,[16] we have learned that a single small dose of tocopherol given to young rats on vitamin E-deficient diet during the late nursing period will significantly delay the onset of testicular degeneration. Since our guinea-pigs were suckled by mothers on a stock diet containing vitamin E, the late appearance of the testicular degeneration may perhaps be ascribed to this fact. Attempts to gain further information on the influence of this milk factor are being made.

Conclusion

Muscular dystrophy developed in guinea-pigs on a vitamin E-deficient diet before the appearance of testicular degeneration. Early degenerative changes were first noted in the testicles after 130 days on the diet, and advanced degeneration was present after 175 days.

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DESCRIPTION OF PLATE

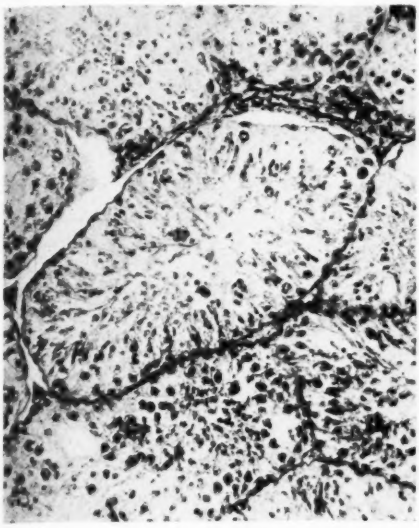
PLATE 45

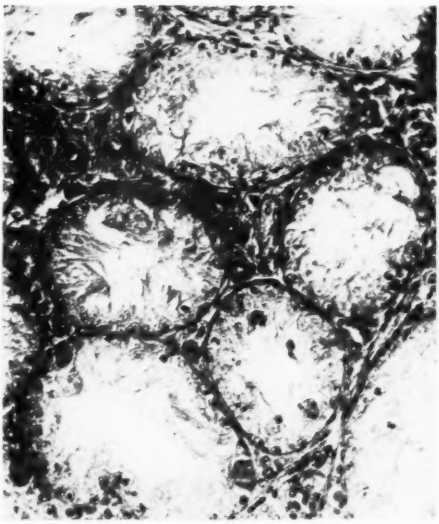
FIG. 1. Guinea-pig 116. Killed after 132 days on diet V B_1. Early degeneration of testis. Hematoxylin and eosin stain. × 190.

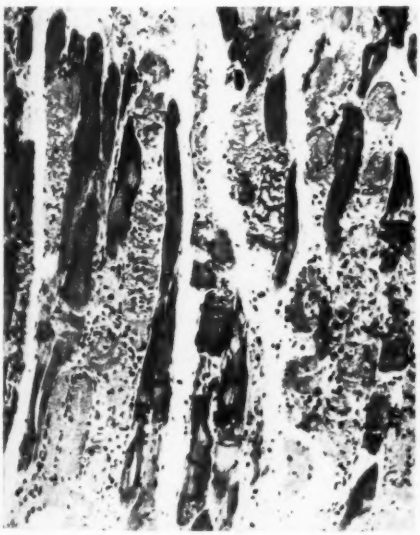
FIG. 2. Guinea-pig 121. Killed after 175 days on diet V B_1. Advanced degeneration of testis. Large pigment cells. Hematoxylin and eosin stain. × 190.

FIG. 3. Guinea-pig 116. Skeletal muscles. Hematoxylin and eosin stain. × 90.

FIG. 4. Guinea-pig 121. Skeletal muscles. Hematoxylin and eosin stain. × 90.







Pappenheimer and Schogoleff

Testis in Vitamin E-Deficient Guinea-Pigs

THE PROTRACTED EFFECT OF A SINGLE DOSE OF dl-ALPHA-TOCOPHEROL ACETATE UPON THE TESTES OF RATS ON VITAMIN E-DEFICIENT DIET *

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In studies of the oxygen consumption of young male rats on a vitamin E-deficient diet, it has been found that a single dose of *dl*-alpha-tocopherol acetate given on the 15th day of life is followed by prolonged lowering of total oxygen consumption as compared with untreated litter mates. This effect is continued until the onset of sexual maturity.[1]

This paper is concerned with a similar protracted effect of a single dose of tocopherol, given to rats on the 15th day, upon postpubertal testicular degeneration.

METHODS

The mother rats were maintained from the time of weaning on a vitamin E-deficient diet which consisted of: casein (commercial), 320 gm.; cornstarch, 400 gm.; lard, 220 gm.; yeast (baker's dried), 100 gm.; salts (Hawke-Oser), 40 gm.; fish oil (Mead's blended), 20 gm. During lactation, 100 gm. of yeast were added to the diet.

When mating was positive, the litter was assured by protecting the mother with 50 drops of wheat germ oil, given within 5 days after mating. To the treated rats, *dl*-alpha-tocopherol acetate (Hoffmann-La Roche) was given by mouth. Dose and day of administration are stated in the tables. In order to make the tocopherol inaccessible to the untreated rats, they were separated from their litter mates for several hours after the administration. Controls on a Rockland pellet diet † supplied data for normal testicular weights. Comparison in individual experiments was always between treated and untreated litter mates; however, the data given in the tables represent mean values derived from different litters.

The right testicle was removed at various ages under ether narcosis; sperm from the vas deferens were examined in Locke's solution for motility or evidence of degeneration, and a histologic study was made of the testis. In grading the lesions, we have followed the stages de-

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† A commercial product containing ground yellow corn, ground hulled barley, ground hulled oats, ground whole wheat, soy bean meal, meat scraps, powdered whole milk, alfalfa meal, NaCl (not iodized), precipitated chalk ($CaCO_3$).

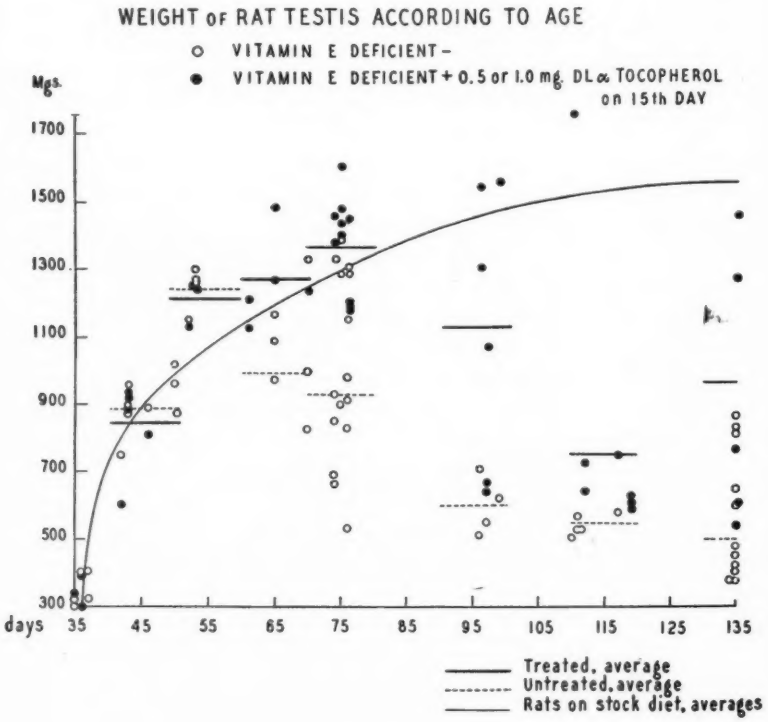
scribed by Mason.[2] After a further period of observation, the second testis was removed, also under ether anesthesia, and the animal killed.

Experimental Findings

Data upon the weights of one testis at various periods after the administration of 0.5 or 1.0 mg. of *dl*-alpha-tocopherol on the 15th day are given in Text-Figure 1, in which are recorded also the weights of one testis of the untreated litter mate controls.

It is obvious that during the prepubertal period treated and untreated rats have about the same testicular weights. A striking difference becomes manifest after the 60th day. The testicular weight of the untreated animals declines, at first rapidly, then more slowly, to reach a final average weight of about 500 mg.

The testicular weight of the treated rats continues to increase in completely normal fashion until about the 80th day. In 4 of 8 animals, maintenance of normal weight continued until the 110th day. Although



Text-Fig. 1.

Text-Figure 1 appears to show occasional overlapping in the later age groups, this was not the case when litter mates were compared. The average final weight of the testis in the treated rats at 135 to 160 days was nearly twice as great as that of the untreated controls, although there were wide individual variations.

It is seen that most of the testicular weights of treated rats fall above a curve based on the testicular weights of rats of the same colony on a stock diet.* This is explained by the better growth and heavier body weight of the litter on our experimental diet.

Plotting testicular weights against body weights has given comparable curves, although the treated animals tended to be somewhat heavier than the controls.

Motility of Sperm

The effect of the tocopherol in extending the period of sperm motility is clearly demonstrated. In 2 of the treated animals, mobile sperm were present on the 163rd day. In not a single animal were degenerative changes noted before the 100th day in the sperm obtained from the vas deferens, whereas in the nontreated rats, swelling and clumping of the sperm were observed in half of the cases during the period of 71 to 80 days, and in practically all cases thereafter.

Fertility

A limited number of fertility tests were made. In the age group from 61 to 76 days, 10 matings with normal females on stock diet† yielded 4 positive results. In 3 cases, the young, in all 27, were observed for 3 months, during which time they showed normal behavior and growth. Matings were attempted with 9 untreated litter mates of the same age group. No sperm were found in the vaginal plugs, and all matings were sterile.

Histologic Changes

The prolonged protective effect of the tocopherol was reflected in the maintenance of normal structure beyond the period in which degeneration of the testis becomes manifest with untreated vitamin E deficiency. This is brought out in Table II and in Figures 1 to 6. Not included are the histologic findings in 9 treated and 10 untreated animals examined between the 35th and 50th days, since there was no detectable difference in the testes at this time. Nor is there any evident

* We are indebted to Dr. Herbert Stoerk for these data.

† During the mating period, males and females were given only the vitamin E-deficient diet.

TABLE I

Motility or Degeneration of Sperm in Treated and Untreated Rats

Age (days)	35 to 50			51 to 60			61 to 70			71 to 81			90 to 100			110 to 120			120+		
	No.	Motile sperm	Degen. sperm	No.	Motile sperm	Degen. sperm	No.	Motile sperm	Degen. sperm	No.	Motile sperm	Degen. sperm	No.	Motile sperm	Degen. sperm	No.	Motile sperm	Degen. sperm	No.	Motile sperm	Degen. sperm
Treated with 0.5 or 1.0 mg. α-tocopherol on 15th day	9	Sperm absent	Sperm absent	3	3	0	6	5	0	13*	10	0	6	1	0	6	0	4	5	2	1
Untreated	10	Sperm absent	Sperm absent	4	3	0	5	0	0	12	0	6	4	0	3	5	0	5	7†	0	1

* The 3 rats with nonmotile sperm had received 0.5 mg. of α-tocopherol. All rats receiving 1.0 mg. had motile sperm.

† Four animals showed complete absence of sperm.

TABLE II

Comparison of Testicular Lesions of Treated and Untreated Rats on a Vitamin E-Deficient Diet

Age (days)	51 to 60				61 to 70				71 to 81				90 to 100				110 to 120				120+			
Testicular lesions*	No.	0 to I	II to III	IV to V	No.	0 to I	II to III	IV to V	No.	0 to I	II to III	IV to V	No.	0 to I	II to III	IV to V	No.	0 to I	II to III	IV to V	No.	0 to II	III to IV	V
Treated with 0.5 or 1.0 mg. α-tocopherol on 15th day	3	3	—	—	6	6	—	—	13	12	1	—	6	3	1	2	6	1	—	5	5	2	—	3
Untreated	4	4	—	—	5	2	3	—	12	2	6	4	4	4	—	—	5	—	1	4	7	—	1	6

* Mason's [8] grading of testicular degeneration:

0 Normal testis.
I Degeneration of spermatozoa.
II Degeneration of spermatozoa and "bead-like" degeneration of spermatids.
III Karyorrhexis of spermatids. Giant cells.
IV Disappearance of giant cells. Degeneration of spermatocytes and spermatogonia.
V Tubules lined with Sertoli cells. Disappearance of all spermatogenic elements.

difference between the 51st and 60th day, corresponding to the onset of spermatogenesis. From this time on, as the table shows, degenerative changes are consistently more advanced in the untreated rats. Even after 121 days, one occasionally finds histologically normal testes in the treated rats.

The lesions were graded without previous knowledge as to whether the individual rat had or had not received tocopherol. When comparisons were made between rats of the same litter, the differences were always consistent.

Effect of Increased Dosage

No systematic study has been made of the comparative effect of varying doses. Through inadvertence, however, 4 rats from three litters received 5 mg. on the 15th day, instead of the usual dose of 0.5 or 1.0 mg. This exercised a more delayed protective effect. In the age period of 90 to 101 days, the average weight of one testis from this group was 1.450 gm. as compared with an average weight of 1.134 gm. in rats which had received the smaller dose. All of the animals had motile sperm, and the testes were histologically normal. At 110 to 125 days, the testicular weight in 4 animals still averaged 1.271, as contrasted with a weight of 0.500 gm. in the untreated controls. Motile sperm were still present in 2, and microscopic examination showed only very early degeneration (Figs. 7 and 8).

Influence of Age at Which Tocopherol Was Administered

It has been shown that tocopherol given during the early period of lactation is relatively ineffective in preventing the onset of muscular dystrophy.[3] This raised the possibility that it might be equally ineffective in delaying the postpubertal testicular degeneration. Experiments bearing on this point are summarized in Table III, from which it is obvious that there is no protective effect whatever when the tocopherol is given in the early lactational period.

Even more interesting is the fact that administration of 1.0 mg. of alpha-tocopherol on the 29th or 30th day—that is to say, immediately after weaning—is less efficacious than when it is given on the 15th day. This is clear from the observations presented in Table IV. Although there is a definite protective effect, it is distinctly less than when the tocopherol is given on the 15th day; and in some litters, the weight of the testis of individual untreated rats exceeds that of the controls.

Discussion

These observations indicate that a single dose of 0.5 or 1.0 mg. of *dl*-alpha-tocopherol acetate given on the 15th day of life produces a

TABLE III

Influence of 1.0 mg. dl-Alpha-Tocopherol Acetate Administered on the 6th to 8th Day of Life on Postpubertal Testicular Degeneration

	Age (days)	No.	Average wt. of 1 testis	Motility of sperm	Degeneration of sperm	Histologic changes (Mason[3])		
						0 to I	I to III	IV to V
			(gm.)					
Treated	71–80	12	1.106	2	5	3	8	1
Untreated	71–80	3	1.177	0	0	—	3	—
Treated	90–100	12	0.608	0	8	—	—	12
Untreated	90–100	3	0.643	0	3	—	—	3

TABLE IV

Difference in Effect Between a Single Dose of dl-Alpha-Tocopherol Acetate Administered on the 15th or on the 29th to 30th Day of Life

	Age (days)	No.	Average wt. of 1 testis	Motility of sperm	Degeneration of sperm	Histologic Changes (Mason[3])		
						0 to I	I to III	IV to V
			(gm.)					
Treated with 1.0 mg. α-tocopherol, 29th or 30th day	71–80	11	1.240	4	0	6	3	2
Treated with 0.5 or 1.0 mg. α-tocopherol, 15th day	71–80	13	1.362	10	0	12	1	0
Treated with 1.0 mg. α-tocopherol, 29th or 30th day	90–100	10	0.774	0	8	1	2	7
Treated with 0.5 or 1.0 mg. α-tocopherol, 15th day	90–100	6	1.134	1	0	3	1	2

significant retardation of postpubertal testicular degeneration in rats maintained on a vitamin E-deficient diet. In agreement with the findings of Mason,[4] we can detect no effect during the developmental period. The onset of spermatogenesis is not delayed in the vitamin E-deficient rats, and the spermatozoa for a short time exhibit normal motility. Beginning at the 60th day, however, there is sharp divergence in the behavior of the testis in treated and untreated rats. The continuing effect of the single early dose of tocopherol is reflected in the greater weight of the testes, in the conservation of sperm motility, in the percentage of fertile matings, and in the histologic structure of the organ. This is consistently true, in spite of wide individual variation in the degree of degenerative change at any given period. A still more evident protection is obtained when the dose is raised to 5 mg.

In contrast to this striking protective effect of tocopherol when given on the 15th day is the complete lack of it when the vitamin is administered on the 6th to the 8th day. This is perhaps less surprising in view of the fact that the early administration fails also to protect against the occurrence of muscular dystrophy.

In the experiments of Mason,[4] the mother and infant rats were transferred on the 14th day from a stock diet containing three times the required amount of vitamin E to a vitamin E-deficient diet. Under these conditions, histologic signs of testicular degeneration began between the 65th and 70th days. This corresponds closely to what has been observed by us in untreated animals. Since Mason's rats presumably received a certain amount of vitamin E during the first 2 weeks of the nursing period, it would seem that a deficiency during the third week is of critical import, and that, as in our experiments, the provision of vitamin E during the early lactational period will not arrest or delay subsequent testicular degeneration. That the third week of lactation is a critical one is further borne out by the fact that when tocopherol is given after weaning it is less effective than when given on the 15th day.

The difference in the average weights of testes between treated and untreated litter mates at the age of 71 to 80 days is thus greatest in those receiving tocopherol on the 15th day, less in those receiving it on the 29th or 30th day, and entirely absent when it is given on the 6th to 8th day. The figures are 0.435 gm., 0.329 gm. and minus 0.061 gm., respectively.

Since over half of the rats treated on the 29th or 30th day had shown previous clinical evidence of muscular dystrophy, it is interesting to inquire whether this may have had a deleterious influence upon the

testicular changes. This is definitely not the case. There is no difference in absolute or relative testicular weights at the age of 71 to 80 days between the animals with and without clinical signs of muscular disease. The lack of correlation between symptoms of muscular disease and testicular degeneration is true also of the untreated animals.

Conclusions

1. Administration of a single dose of 0.5 or 1.0 mg. of *dl*-alpha-tocopherol to the offspring of vitamin-depleted mother rats delays the onset and retards the course of postpubertal testicular degeneration.

2. Administration of 5 mg. on the 15th day produces a still greater protective effect.

3. Administration of 1 mg. on the 6th to 8th day is without effect.

4. Administration of 1 mg. on the 29th to 30th day affords less protection than when given on the 15th day.

We are greatly indebted to Dr. R. D. Shaner of Hoffmann-La Roche, Inc., Nutley, N. J., for the tocopherol used in these experiments.

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Description of Plates

Plate 46

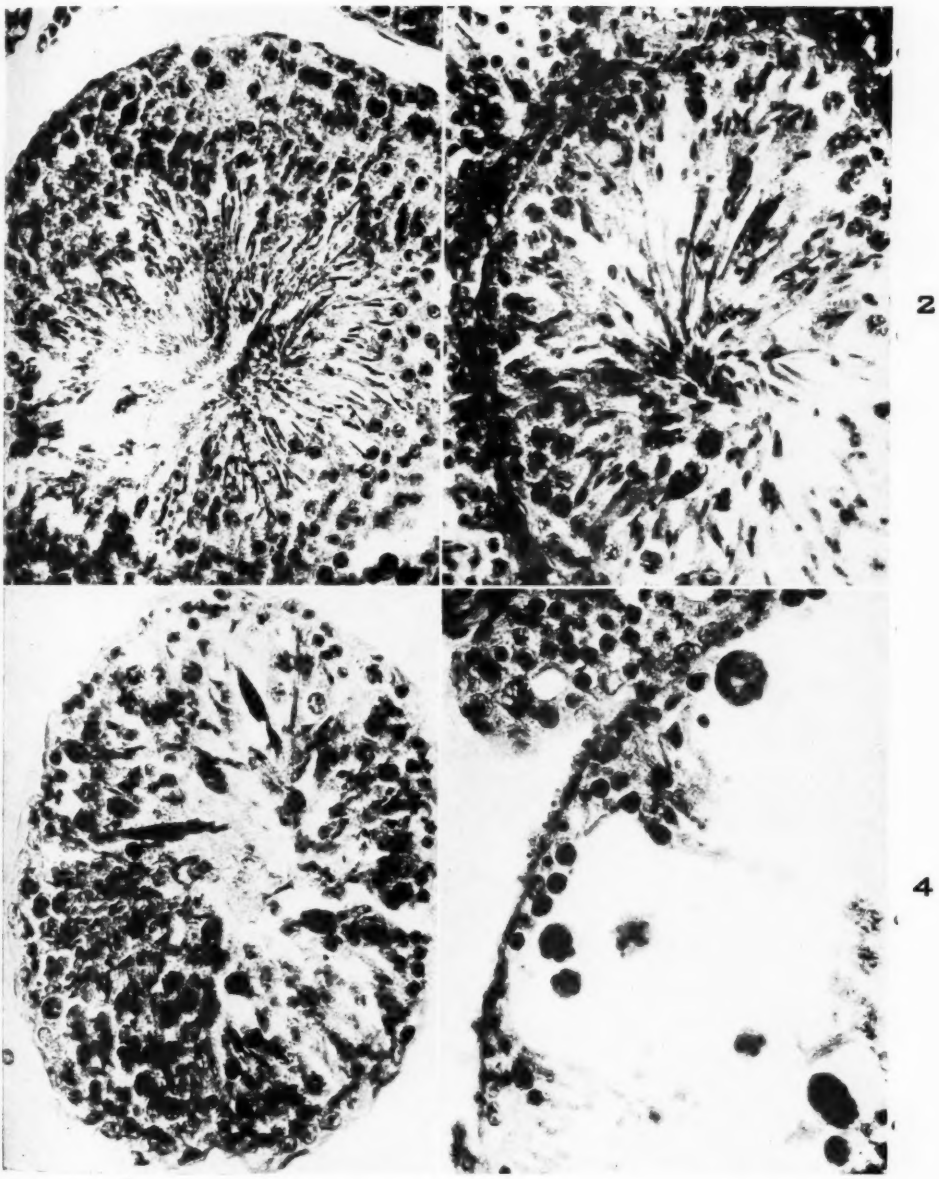
Each pair of figures, beginning with Figures 1 and 2, represents testes from litter mates. Sections are stained with Weigert's iron hematoxylin and eosin stain. × 490. See Table II for Mason grading.

Fig. 1. 65 days. 1 mg. *dl*-alpha-tocopherol on 15th day. Testicular weight, 1.482 gm. Mason stage 0.

Fig. 2. 65 days. Untreated control. Testicular weight, 1.086 gm. Mason stage I.

Fig. 3. 75 days. 1 mg. *dl*-alpha-tocopherol on 15th day. Testicular weight, 1.378 gm. Mason stage I.

Fig. 4. 75 days. Untreated control. Testicular weight, 0.936 gm. Mason stage III.



Kaunitz, Pappenheimer and Schogoleff

Effect of *dl*-Alpha-Tocopherol Acetate upon Testes

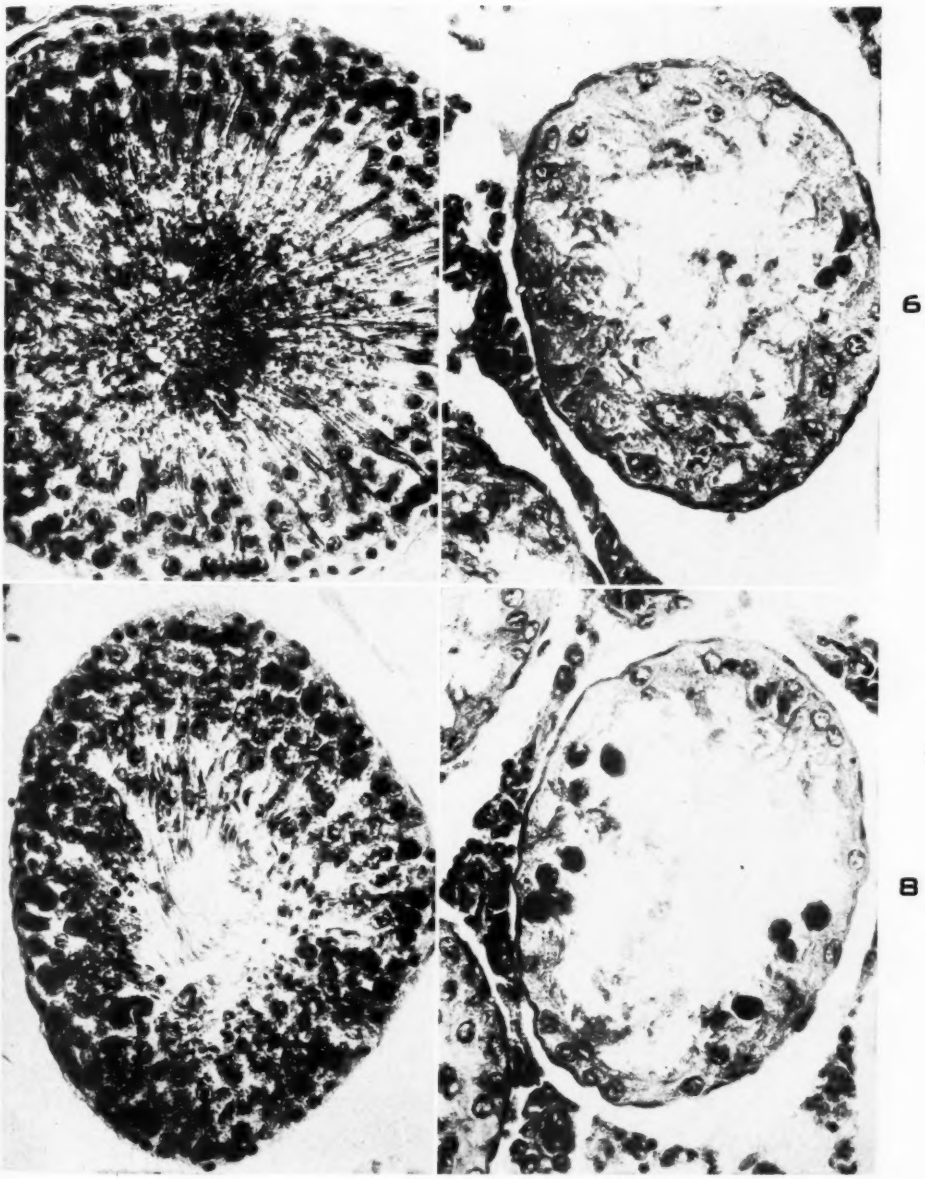
PLATE 47

FIG. 5. 97 days. 0.5 mg. *dl*-alpha-tocopherol on 15th day. Testicular weight, 1.071 gm. Mason stage 0.

FIG. 6. 97 days. Untreated control. Testicular weight, 0.549 gm. Mason stage V.

FIG. 7. 121 days. 1 mg. *dl*-alpha-tocopherol on 15th day. Testicular weight, 1.491 gm. Mason stage I.

FIG. 8. 121 days. Untreated control. Testicular weight, 0.594 gm. Mason stage V.



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Effect of *dl*-Alpha-Tocopherol Acetate upon Testes

CHRONIC EQUINE ENCEPHALITIS *

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As a result of recent investigations, it has become evident that of all the forms of acute encephalitis, equine encephalitis, especially the western strain, produces the most characteristic alterations in the brain. Many investigators accept these changes as pathognomonic of this disease even in the absence of further corroborating evidence (Baker and Noran,[1] Weil and Breslich[2]). During the past few years evidence has accumulated indicating that in this disease permanent and progressive residual lesions may result, especially in children. Most of the recorded cases have been limited to clinical reports in which the symptoms and signs have been strikingly similar from case to case. The most constant manifestations have consisted of epileptiform seizures, progressive mental deterioration and enlargement of the ventricular system as demonstrated by pneumograms. The structural changes responsible for such a picture were unknown until a recent publication from this laboratory.[3] The case described was that of a child, 3½ years old, who died approximately 3 years after an attack of equine encephalitis, and who, throughout the course of illness, showed a very high neutralizing titer against the western equine virus. The brain presented most unusual findings. We had never observed similar changes in any other neurologic disorder and to us these changes appeared to be pathognomonic of chronic equine encephalitis. These changes consisted of an extensive cystic degeneration of various selected regions of the cerebral hemispheres. The involved areas were replaced by numerous cavities separated merely by thin layers of gliotic brain tissue. A large portion of the brain showed marked ganglion cell alterations with patchy and diffuse glial proliferation. The most striking features were the vascular changes which consisted of endothelial proliferation and extensive vascular calcification even to the extent of completely occluding some of the vessel lumina. Focal areas of demyelinization, so characteristic of the acute illness, also were observed. Although no single feature listed was in itself diagnostic of this condition, the combination of gross and microscopic findings certainly was unique and suggestive of illness of a new type.

Shortly after our original publication on this subject, another case

* This study was aided by a grant from the Research Funds of the Graduate School of the University of Minnesota Medical School.

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came to my attention in which the pathologic findings were identical with those observed in our proved case of chronic encephalitis. Even though there was no way of identifying this case definitely by means of serum neutralization, still the clinical course and especially the pathologic features were so characteristic as to leave little doubt as to the correct diagnosis.

Report of Case

R. J. (N1754), a white woman, 49 years of age, died following an accident in which she had been struck by an automobile while crossing a street. She sustained a crushing injury to the chest, compound fractures of the right tibia and fibula, a laceration of the scalp and multiple fractures of the pelvis. Despite transfusions of blood and plasma she remained in shock and expired 26 hours later.

The history of the earlier part of her life was incomplete for the only informant available was a sister 11 years younger than the patient.

At the age of 10 days the patient was known to have developed a severe illness diagnosed as brain fever which produced a paralysis of the left side of the body. Recovery, however, was sufficient to allow her to walk at an early age, but a hemiparesis persisted and the extremities on the left became noticeably atrophic. The left arm was held in a flexed position and the left foot dragged in walking. Nonetheless, she became able to walk for long distances. Her left upper limb remained much more severely involved in spite of the fact that she used it a great deal in an attempt to increase its strength.

Early in her childhood epileptiform seizures appeared and recurred at frequent intervals. They were described as sudden attacks of unconsciousness associated with a tonic spasm of the entire body and foaming at the mouth. They were often considerably prolonged and on a number of occasions inhalations of chloroform were required to halt them. At the age of 25 years these attacks abruptly ceased. Between the ages of 30 and 40 years, however, three apoplectiform attacks made their appearance. These were characterized by a sudden loss of consciousness of about 24 hours' duration followed by a protracted period of profound weakness which would persist for about 1 week. Recovery then would be rapid without apparent neurologic residuals.

A number of years before her death she had undergone an enucleation of her left eye following a panophthalmitis, and on another occasion had fractured a clavicle. Menstruation had never occurred. Except for a few minor illnesses of no apparent significance she was always considered to be in excellent health.

She started in school at the age of 8 years. By the time she reached the second grade it was apparent that she was subnormal intellectually and would be unable to continue. She received special tutoring at home for several years with discouraging results, but she did learn to read and write a little. Her accomplishments along these lines, however, were less than those of the informant's 7-year-old son. She led an extremely sheltered life with her relatives. Her chief activity consisted in helping with some of the less complicated household duties. With even the more simple tasks she required constant supervision and was never able to assume any responsibility. Throughout her life her behavior remained at a childish level in that she continued to be interested in playing with dolls and showed an emotional immaturity manifested by occasional temper tantrums.

During the past several years of the patient's life her relatives noticed a gradual decline in her intellectual functions. Her memory for recent events steadily failed so that after only a very brief period she was unable to recall where she had placed certain familiar articles. In addition, various things that were formerly put in a

definite place would be found in different locations every time she used them. Although her mental capacity was always low, her acquaintances felt that a mild but very definite intellectual failure had recently taken place.

Autopsy Findings

There was a laceration of the scalp about 3 cm. in length with slight subcutaneous hemorrhage in that region. Fractures of the second to ninth ribs were found posteriorly on the right, which had caused some laceration of the parietal pleura. There were fractures of the pubis and ileum on the right which had produced a moderate extravasation of blood into the pelvis. There were no fractures of the cranial vault or evidence of dural bleeding.

Gross examination of the brain revealed a marked discrepancy in size between the two cerebral hemispheres. The right, which was normal in appearance, was nearly twice as large as the left. Very striking alterations were evident in the left frontal and temporal lobes. The entire left frontal lobe was remarkably shrunken and atrophic, its tissues appearing thin, opaque, and fluctuant to palpation. Only in scattered areas were remnants of the former convolutional pattern discernible. Except for a small portion of the anterior pole, there was a complete destruction of the left temporal lobe. This region of the hemisphere was represented merely by a small depression under the parietal lobe which was covered by a thin layer of fibrous, structureless tissue. The anterior and posterior central gyri, as well as the parietal and occipital lobes of this hemisphere, demonstrated a normal convolutional pattern although the individual gyri were smaller than the corresponding convolutions on the right. The left anterior cerebral artery was notably smaller than that on the right. Otherwise the cerebral arteries disclosed no abnormalities. The brain stem and cerebellum appeared normal.

Coronal sections evidenced an almost complete destruction of the left frontal lobe which was replaced by numerous small cysts covered by a thin rim of fibrous tissue (Fig. 1). In only a few very small disseminated areas were foci of brain tissue recognizable and even in these identification was questionable. The entire left lateral ventricle was considerably dilated. This dilatation was most marked anteriorly where it appeared to be the result of extensive tissue destruction. The basal ganglia of this hemisphere were displaced downward but displayed a normal architecture. The left thalamus, however, was definitely smaller than that of the opposite hemisphere. With the exception of the anterior pole, the left temporal lobe had been converted into a thin fibrous membrane which in places was semitranslucent. In the adjacent

portion of the left occipital lobe were numerous tiny cystic areas. The right cerebral hemisphere as well as the brain stem and cerebellum showed no gross variations.

Microscopic sections revealed a similar picture in the left frontal and the left temporal lobes. There was an extensive replacement of the parenchyma in these regions by numerous glia-lined cysts of various sizes. Many of the cavitations contained scattered fat-granule cells and were traversed by strands of intertwining neuroglial and mesodermal elements. A few of the very small cystic formations had undergone a relatively complete glial repair. The cysts were frequently separated by very thin strands of gliotic tissue and were covered merely by a thin band of cortex (Fig. 2). The cortical gray matter disclosed a complete devastation of its ganglion cells and all but the outer layers were replaced by the cystic degeneration. The remains of the underlying white substance showed a relatively complete destruction of myelin sheaths and axis cylinders. Throughout all this devastated tissue there was a profound proliferation of the macroglia. In many regions the gliosis was principally cellular with very little production of astrocytic fibers, while in other areas the altered tissues were completely replaced by a dense meshwork of coarse, intertwining neuroglial fibers. In a few areas dense, acellular scars had resulted. Scattered diffusely through this proliferating glial tissue were numerous globules of calcification, often arranged in focal collections. These globules were comprised of densely packed calcium granules corresponding in size to the small cortical blood vessels. Corpora amylacea were only occasionally observed.

The small blood vessels of the frontal and temporal regions, especially the arterioles and capillaries, demonstrated swelling and proliferation of their lining endothelium, often with relatively complete occlusion of the lumen. Often all of the elements of the vessel wall had proliferated, resulting in extreme thickening of the wall and obliteration of the lumen. A large number of these vessels had become hyaline, creating an appearance of a fibrous mass. In vessels of this latter type, calcium granules were found most often around the intima but often replaced the entire vessel so that the vascular structure could not be identified. The small veins displayed considerable adventitial proliferation and many were encircled by mononuclear cells, mainly lymphocytes. In numerous small vessels this mononuclear infiltrate formed thick collars filling the perivascular spaces. Focal collections of leukocytes, consisting of both lymphocytes and polymorphonuclear leukocytes, were also found in a few disseminated areas intermixed with a small number of astrocytes.

In the regions of the cerebrum that were grossly normal, less prominent variations were seen. These were most pronounced in regions adjacent to the areas of gross tissue destruction, but were also found throughout the entire cerebrum. Alterations of the cortical neurons had occurred only in disseminated areas. The most constant neuronal change consisted of pyknosis and shrinkage of the cell body, but a few cells were swollen and chromatolytic with occasional ghost cell formations. These alterations frequently occurred in the vicinity of a small vessel showing extensive endothelial proliferation with occlusion of the lumen. Frequently there was a dropping out of some of the neurons and in certain cortical areas there was an increase in astrocytes. Scattered throughout the gray matter of the occipital region there were, in addition, large globules showing concentric rings of calcification reminiscent of the vascular calcification previously described.

Within the white matter of the grossly normal regions of the hemispheres, there were numerous scattered areas of focal demyelinization which were generally not associated with any glial increase. These were irregular in outline and quite sharply demarcated from the surrounding tissue. In the subependymal region the demyelinization tended to be more diffuse. Glial nodules, although not common, were occasionally seen. A few subcortical vessels displayed a perivascular lymphocytic infiltrate, while the walls of a few others were invaded by neutrophils. This latter change was seen only in selected instances.

In all of the basal nuclei nerve cell alterations were prominent. The ganglion cells were frequently swollen and their cytoplasm contained relatively large amounts of granular lipofuscin pigment. The nuclei were eccentric and often fragmented. Ghost cells were not uncommon. Large and small focal areas of demyelination were found. In these areas the nerve cells were shrunken, pyknotic and even fragmented. A few glial nodules were seen in the pallidum. Occasional perivascular infiltrates were also encountered in the corpus striatum, but they were infrequent.

In the brain stem and cerebellum the alterations were mild and of a patchy nature. Regressive neurocellular alterations were found as well as irregular areas showing myelin degeneration. Well formed collars of lymphocytes filled the perivascular spaces of a few vessels (Fig. 3).

Discussion

It seems fairly evident that the pathologic features in the present case are unusual and correspond in every detail with those already described in a proved case of chronic equine encephalitis. It is felt that such

characteristic cerebral alterations are in themselves sufficient for diagnosis, even in the absence of corroborating evidence such as neutralizing antibodies in the serum or the occurrence of the illness during an epidemic of the disease. Even though equine encephalitis has only recently been definitely identified in both the horse and man, it is well recognized that this disease has existed in this country for many years and the clinical syndrome has been described in many epidemics among horses. In view of the prevalence of this disease, at least among the equine population, it would seem inevitable that human infection should occur, especially since it is now known that man is susceptible to this virus. No doubt many cases of human infection have been occurring for years, but have gone unrecognized because of the lack of adequate means of identification in the laboratory. Since this disease does produce chronic and progressive sequels, we would expect to encounter, at least occasionally, cases in which the brain reveals findings indicative of the original infection.

The present case is most instructive because histologically there appeared to be every evidence, not only of chronic damage, but also of an active acute process as indicated by the foci containing polymorphonuclear leukocytes, the extensive perivascular monuclear collections and the vascular endothelial increase. That such an active process was still in progress was also strikingly revealed by an analysis of the clinical course of the illness. Certainly both the increasing mental deterioration and the periodic apoplectiform seizures pointed to activity. In addition, the disappearance of the epileptogenic focus after 25 years suggested the presence of a progressive destruction of cortical tissue. These observations of a process both clinically and, especially, pathologically active for over 40 years after the onset of an encephalitis are most amazing and strongly influence our concept of virus infections. It would appear that in some virus infections, the recovery from the acute illness merely indicates an ability of the host to prevent the further spread of the virus, but that the noxious agent is not destroyed and is capable of again spreading as soon as the host's resistance is reduced. At least in equine encephalitis, one questions whether the virus is ever destroyed, once it invades the human organism. That this concept may be true is further suggested by the observations of many investigators that in this disease, neutralizing antibodies can be observed in high titers many years after the acute illness.[4]

Conclusions

1. A case of chronic equine encephalitis is presented.

2. Both the gross and microscopic lesions were identical with those previously described in a proved case.

3. Grossly the brain revealed an extensive cystic degeneration of the left frontal and temporal lobes. Microscopically there was a widespread parenchymal degeneration with focal areas of inflammatory infiltrate. Many vessels were occluded by an endothelial proliferation and deposition of calcium.

4. A pathologic picture is described which is seen in no other condition and is, therefore, regarded as pathognomonic for chronic equine encephalitis.

5. The histologic evidences of active inflammation as well as the clinical course demonstrate that the western virus may remain active for many years in equine encephalitis.

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[*Illustrations follow*]

DESCRIPTION OF PLATE

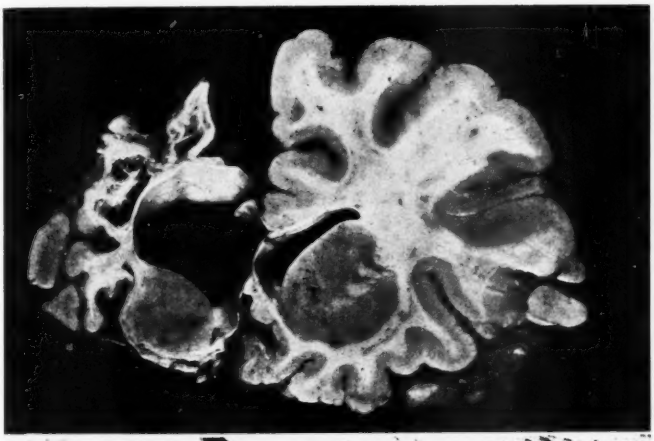
PLATE 48

FIG. 1. A coronal section demonstrating the extensive destruction of the left frontal lobe. The ventricle is extremely dilated and the remaining tissue harbors large cystic areas.

FIG. 2. A thin band of frontal cortex overlying a large cystic area. There is a complete devastation of ganglion cells and a diffuse glial proliferation. Small globules of calcification are scattered throughout the cellular areas. Hematoxylin and phloxine stain. × 150.

FIG. 3. A perivascular accumulation of lymphocytes resembling that seen in acute equine encephalitis. Hematoxylin and phloxine stain. × 375.

1



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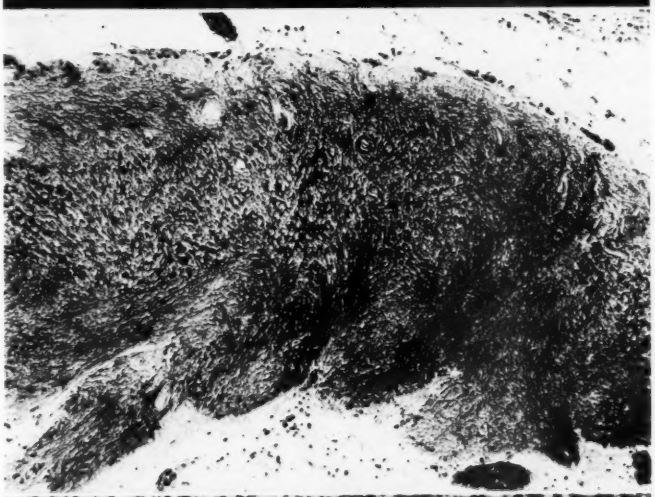

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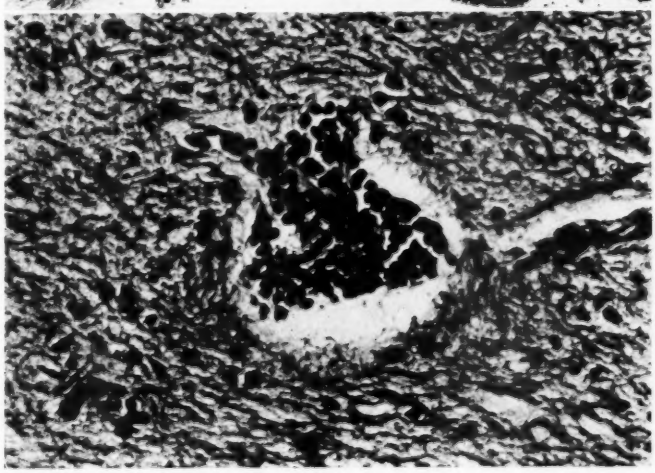

MENINGOCOCCAL ENDOCARDITIS IN IMMUNIZED HORSES*

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The essential condition in the pathogenesis of bacterial endocarditis is one of localization of the incitant. Bacteria, irrespective of any special selectivity of the microorganisms for the particular tissue, may passively localize on valvular endothelium that has been predisposed by some injury. After sensitization to an antigen, an animal develops a hyperergy to subsequent invasion of that antigen, or even other antigens, that is manifested by morphologic changes in the endothelium and certain other tissues. The injured tissues are thus predisposed to bacterial localization.[1-5] The animal under immunization in various stages of sensitization presents precisely such conditions for study.

Among 110 horses under immunization with meningococci, 14 developed endocarditis. All had been given living cultures of meningococci of groups I-III and II intravenously.[6] One horse had previously received diphtheria toxin and 3 horses had also been injected with meningococcal filtrate, either subcutaneously or intravenously, at some time during immunization.

CLINICAL RÉSUMÉ

A review of the records of the 14 horses with endocarditis and the clinical and autopsy notes by Drs. Charles A. Griffin and Cyrus P. Brose, Veterinarian-Bacteriologists on the staff, are briefly summarized to outline the significant symptom-complexes and anatomic changes found at autopsy. The 14 horses varied in age from 7 to 26 years, averaging 17 years, and were under immunization for from 5 to 26 months, an average of 14.5 months.† Following the injections, all horses that received living microorganisms had variable reactions characterized by trembling, dyspnea, weakness, tachycardia, and weak pulse. Injections of toxic culture filtrates usually induced fever and subcutaneous edema. The animals developing endocarditis presented a progressive symptom-complex of fever, weakness, loss of weight, subcutaneous edema, and weakness of the extremities. In 9 of the 12 animals with fever, meningococci were obtained in the blood cultures

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† The entire group of 110 horses were of the same age range. Fifty-four horses (50 per cent) were under immunization for from 6 to 9 months and 46 (40 per cent) from 20 to 24 months. Four horses were immunized for from 36 to 48 months and 1 animal has been yielding serum of high antibody titer for 10 years.

taken when the febrile reaction failed to subside. The febrile reactions with bacteremia persisted from 5 to 105 days, averaging 38 days, when the horses either were destroyed or died. With few exceptions, the horses with bacteremia had produced sera of high potency. A cardiac murmur was heard in 8 horses, appearing as early as the 5th month and as late as the 30th month of immunization and persisting as long as 100 days. Nine animals had subcutaneous edema exclusive of that induced by injections of filtrate. Cyanosis, epistaxis, and excessive perspiration were each encountered once. Weakness of the hindquarters occurred in 10 and definite lameness appeared in 5 animals.

Autopsies were performed on all 14 horses. Tissue was fixed in Zenker's fluid and formaldehyde solution. Sections were stained with hematoxylin and eosin and with phosphotungstic acid hematoxylin and, for demonstration of microorganisms, by the Brown-Brenn method [7] and with carbol thionin.

Gross Anatomic Observations

All 14 horses had endocardial valvular lesions of the left side of the heart. The right side was not involved. In twelve hearts, the aortic valve bore vegetations varying from a small verruca to an ulcerative mass, 3 cm. in diameter. All of the cusps of three aortic valves were involved. The mitral valves of three hearts had vegetations and in three more hearts the mitral cusps were thickened and edematous. In 12 horses, cultures of meningococci were recovered from affected valves. Adherence of a mitral vegetation to the ventricular wall and a vegetation on chordae tendineae were encountered. Neither the symptoms nor even the condition of the horses indicated the extent of the valvular lesion.

The gross pathology of these horses was further characterized frequently by vascular thromboses and accompanying visceral infarction. The autopsy notes record that the following vessels were thrombosed: pulmonary artery, twice; pulmonary veins, twice; renal artery, once; renal veins, twice; splenic artery, four times; splenic vein, once; hepatic artery, twice; hepatic portal vein and tributaries, four times; colic artery, once; and mesenteric artery, four times. Infarcts were observed in the lung, once; in the kidneys, three times; in the spleen, twice; and in the liver, once.

In microscopic sections from one horse, infarcts in the lung, liver, and kidney parenchyma with attendant arterial thrombi were found. Tissue sections from a second horse contained hepatic and renal arterial thrombi, while splenic and renal infarcts were demonstrated in stained tissues of a third animal.

Two hearts presented definite myocardial hypertrophy, and gross myocardial scarring was noted in one cardiac apex. Pneumonic consolidation was found at autopsy in three horses. Enlargement of the spleen, a frequent development in immunized animals, was found only four times. There was a varying amount of liver damage. Twice the hepatic capsule was ruptured and this was accompanied in one horse by fatal hemoperitoneum and in another by massive subcapsular hematoma. With thrombosis of the colic artery, gangrene of the colon occurred. The kidneys were usually swollen, edematous and pale. Subcapsular hemorrhages were frequent and purulent material was found in the pelvis three times.

Results of Bacteriologic Studies

As a result of study of cultures recovered from these horses from the blood stream or at autopsy, it was reported [6] that: "Meningococci were isolated from eleven horses which developed endocarditis during immunization against several meningococcal strains of groups I-III and II. The cultures from ten horses were group I-III and those from eight of these animals resembled closely one of the stock strains used in immunization. The culture from the eleventh horse was mixed, but group-II microorganisms predominated. No changes occurred in the agglutinative or precipitative activities of cultures isolated repeatedly from individual horses. Of those tested, the cultures were, in general, of low virulence for mice, lacked well-defined capsules, and possessed a marked capacity to survive in sodium-chloride solutions."

Meningococci of group I-III were also recovered from two other horses.

Histopathologic Findings

Heart

Valves. Although the predominant lesion on the valves was a macroscopic ulcerative vegetation, the endocardium exhibited certain characteristic changes: initially, a swelling of the endothelial cytoplasm with swelling and elongation of the nuclei. This endothelial edema gave rise to a separation of the endothelial layer from the subendothelial tissue leading to wrinkling, fragmentation and, finally, desquamation of the endothelium. In some areas, gram-negative diplococci could be demonstrated on the swollen endothelium among the desquamating cells and in the superficial portion of edematous subendothelial tissue. The denuded surface occasionally showed a fibrin layer containing endothelial cells, blood elements and bacteria. Buds of endothelial cells were noted in the reparative processes of this denuded area. These

manifestations were more strikingly demonstrated in a horse that had been treated with sulfanilamide, which prolonged the course of the disease, clearing the bacteremia which reappeared regularly on abandoning treatment.

In other areas, a bare subendothelial layer showed fibroblastic proliferation with fibroblasts tending to form a pseudopalisade surface layer of spindle-shaped cells with fibrillary processes separated by intercellular edema. This reaction often presented a burgeoning of fibroblasts forming papillary projections. The fibroblastic tissue was infiltrated by numerous mononuclear cells. This pseudopalisade layer of tissue, likewise, may be the repository for circulating blood elements and bacteria.

The fibroblastic wall, reparative and defensive, further changed into a loose fibrillary structure with atrophic cells through a process resembling mucoid degeneration. The fibrous verrucae became covered with endothelium or accumulated fibrin and blood elements. Some were hyalinized, vascularized and fibrosed with resultant scarring of the valve.

At any stage from the initial edematous endothelium to the scarred verruca, successful bacterial colonization, with or without antecedent thrombotic processes, may give rise to the well known ulcerative vegetation. Frequently the transformation from swollen endocardium to ulcerative vegetation was seen as one progressed from the base of the valve to the distal vegetative lesion. In many places the transformation of the pseudopalisade layer of subendothelial tissue into granulation tissue occurred adjacent to the thrombotic vegetation proper. The fibrochondroid plate was edematous and there was moderate to marked thickening of the arteriolar walls due to medial and intimal hyperplasia. However, hemorrhagic foci of the endocardium and of the blood vessels of the valves, such as Wadsworth [8] noted in horses under pneumococcal immunization, were not observed in these valves. No thrombosis of the blood vessels of the valves was seen and no intravascular bacteria. Bacterial stains revealed bacteria only on the surface of the damaged valve or in the superimposed thrombotic mass. Microorganisms were not seen in the depths of the subendothelial tissue. The advanced vegetation consisted of a fibrin mesh containing disintegrating erythrocytes, cell débris, blood pigment, and irregular strata and clumps of polymorphonuclear leukocytes and gram-negative cocci. On the surface of the vegetation, colonies of bacteria were large and numerous; this is in contrast to the paucity of pneumococci Wadsworth [8] observed in vegetations of pneumococcal endocarditis. The bases of these meningo-

coccal vegetations showed hyalinization and fibroblastic invasion. This granulation tissue contained occasional small abscesses and considerable round-cell infiltration.

Myocardium. The ventricular and auricular myocardial damage varied. Interstitial edema was not uncommon. Old, minute, fibroid infarcts, scattered areas of myocardial degeneration and atrophy with fibrous replacement, and some arteriolar sclerosis were occasionally found. In one horse, an acute, purulent, interstitial myocarditis was observed at autopsy.

Lungs

In the lungs of two horses, arterial thrombi were demonstrated. A large artery was seen to contain a thrombus extending into tributaries and leaving empty distal vessels. The endothelium was swollen, vacuolated and ragged, with some interruption of the media. Occasionally bronchitis, peribronchitis and patchy bronchopneumonia were encountered.

Liver

The hepatic damage varied from a chronic venous congestion to moderately severe parenchymatous degeneration, largely central in distribution. One liver showed a periportal cirrhosis accompanied by bile duct proliferation; one duct containing purulent material in the lumen. Another liver with a large venous thrombus had approximately one-third of the parenchyma destroyed and replaced by a suppurative process and fibrosis. These changes are in general agreement with the observations of Wadsworth, Hyman and Nichols [9] on the livers of 41 horses immunized with tetanus and diphtheria toxins and with meningococcal, pneumococcal and streptococcal cultures; in fact, they included in their studies of the lipid content of such livers two horses with meningococcal endocarditis (no. 287 and no. 347).

Spleen

There were numerous thrombi and infarcts, and several spleens showed reticular hyperplasia with multinucleated giant cells.

Kidneys

Most pronounced were the severe and general interstitial changes in the kidneys consisting of focal and diffuse infiltrations with mononuclear cells, accompanied by generalized advanced tubular degeneration with a variety of casts, largely leukocytic, epithelial and erythrocytic. In some kidneys, extensive fibrosis existed. The kidneys of six horses

showed definite glomerular damage. This varied from capillary hyperemia, swelling and vacuolation of the endothelium, exudation and hemorrhage into Bowman's space, to fibrosis and hyalinization of the glomerulus, capsular thickening and crescents. Thrombi of the renal arterioles were found in one animal and ischemic renal infarcts in two horses.*

Discussion

The lesions in the valves of those horses under immunization that developed meningococcal endocarditis appear to correspond in the initial as well as in the later stages with those reported in experimental studies on the development of endocarditis without antecedent mechanical trauma in previously sensitized animals that have been given living microorganisms. Nedzel [2] and Keefer [11] have reviewed the literature on experimental endocarditis. As early as 1919, Wadsworth [8] described the endocardial valvular lesions in horses under pneumococcal immunization, and, more recently, horses used in serum production were not infrequently found to have endocarditis.[12, 13] The occurrence of arterial and venous thrombi in these horses supports experimental [3] and clinical [4] observations in which thrombosis has been found to be associated with vascular endothelial changes in the sensitized or immunized subject.

Since damage to the valves and blood vessels occurred in the absence of mechanical trauma, it may well be that the endothelium of such horses is altered in the course of immunization in such a manner that it is a favorable focus for bacterial localization or thrombus formation. The altered endothelium may become more vulnerable to the toxic bacterial products that prepare the way for subsequent colonization of the microorganisms, as suggested by Wadsworth [8] 25 years ago in his study of endocarditis in horses under pneumococcal immunization. Judging from the type of lesions induced, many bacterial toxins have a selective action on tissues. One group, exemplified by streptococcal toxins, is essentially an endothelial poison and gives rise to hemorrhage in the tissues. As Wadsworth has stated: [14] "Tissue susceptibility, possibly specific sensitization, is the underlying condition determining the action of the toxin of the different streptococci, as was very strikingly illustrated, in one instance, by the development of hemorrhagic purpura in the course of a human infection of several years' duration." Toxic vascular damage is particularly suggested with the pneumococcus for Wadsworth [8] noted hemorrhagic foci in the endocardium and in the

* Dr. Joseph Schleifstein has made an extensive study of the nephritic changes; they were reported in abstract [10] and will be published.

valvular vessels. Such lesions were not seen in these studies of meningococcal endocarditis. The serum of the horses with pneumococcal endocarditis had a high antibody titer; that of horses with meningococcal endocarditis likewise was generally of high potency.

The normal cardiac valve of the horse contains blood vessels through which bacteria may gain access to the damaged endocardium. It is extremely difficult to demonstrate bacteria morphologically in the blood even though a bacteremia is present. Bacterial stains of these sections failed to reveal intravascular microorganisms; hence the difficulty of excluding access to the endocardium through the blood vessels. However, the distribution of bacteria as observed in these studies suggests localization from the blood bathing the valve surfaces.

Endothelial changes, similar to those observed in horses with meningococcal endocarditis, were noted in a very thin aortic valvular plaque from a horse under pneumococcal immunization. A rabbit, after having only six injections of killed pneumococcal vaccine, had a mitral valve with large edematous endocardial verrucae, as yet devoid of bacterial or thrombotic elements. The altered behavior of host tissues toward specific bacterial antigens frequently leads, first, to a stage of hypersensitivity and, later, to a stage of insusceptibility to infection and immunity, which may fluctuate in the same host. Thus, we have different phases and degrees of susceptibility to infection which must be taken into account in considering the influence of the immunizing process on the development of endocarditis. The early stages should be studied in order to determine the interrelation of endothelial alterations and bacterial endocarditis occurring during immunization of animals.

These observations on the different stages of the development of endocarditis in animals under immunization appear to correspond with those reported by others in experimental and clinical studies. In these horses immunized with the meningococcus, endothelial damage appears to be the primary stage, leading to inflammatory reactions, thromboses and localization of the bacterial incitant. This endothelial damage may be associated with varying stages of susceptibility developing in the course of immunization.

Summary

Among 110 horses under immunization with the meningococcus, 14 animals developed endocarditis; multiple arterial and venous thrombi were frequently found at autopsy. From the histologic studies of the autopsy material of these 14 animals it appears that the initial stage in the development of the endocarditis is edema and swelling of the valvular endothelium with wrinkling, roughening, and finally desquama-

tion of endothelial cells. This leads to inflammatory cellular reaction and a reparative process, or to thrombosis and localization of bacteria that culminates in the advanced ulcerative vegetation. The tissue changes suggest that in the course of immunization alterations may occur in the endothelial tissues, leading to injury that predisposes to bacterial localization and subsequent endocarditis.

I wish to express my gratitude to Dr. A. B. Wadsworth not only for suggesting this study but for his invaluable guidance.

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CULTURAL CHARACTERISTICS OF A HEMANGIOENDOTHELIOMA*

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The hemangioendothelioma is a rare variety of malignant neoplasm characterized by the formation of anastomosing vascular tubes and atypical endothelia. In another communication [1] one of us (A. P. S.) has reported 18 cases of hemangioendothelioma illustrating its variations in growth rate and morphology. One of those cases has been studied by the method of tissue culture and it is our purpose here to describe the details of that investigation. The tumor is of unusual morphological appearance but its behavior *in vitro* leaves little doubt that it represents an authentic variant of this interesting vascular tumor type.

CASE HISTORY

The patient was a colored male, 28 years old. The growth first appeared as a marble-sized lump in the left calf 3 years before operation. It did not seem to increase in size until 9 months before treatment when the calf began to swell and became painful. Aspiration 4 months before had yielded only blood. Examination on admission to the hospital showed a diffuse enlargement of the left calf which measured 47.5 cm. in circumference while the right calf was 36 cm. The swelling seemed to be due to a deep-lying fusiform tumor of vague outline. It was suggested that it lay deep to the soleus muscle. The periosteum of the fibula seemed roughened on x-ray examination, suggesting that the tumor might be attached to it. The left calf felt warmer than the right. Operation was performed on March 13, 1942, under the supervision of Dr. C. D. Haagensen. The tumor was first approached through a posteromesial incision but so much bleeding was encountered when its vicinity was reached that a specimen for biopsy was not taken here but a second posterolateral incision was made. After the lateral head of the gastrocnemius muscle was partly divided and the soleus retracted mesially, it was possible to obtain tissue for biopsy, although bleeding was profuse. A frozen section was interpreted as a malignant tumor but its exact nature could not be stated. Amputation was then carried out through the lower third of the femur. One year later there was no evidence of metastasis or recurrence.

After the leg was dissected, it was found that the flexor digitorum longus and flexor hallucis longus muscles were the site of a tumor which measured 16 cm. from above downward, 13.5 cm. in greatest transverse diameter and 6 cm. from before backward. It was exceedingly vascular and contained large spaces filled with viscous, black, bloody fluid in some areas while elsewhere the relatively homogeneous, soft, pink tissue was speckled with countless tiny red dots. The tumor had also invaded the soleus and peroneus brevis muscle. It was sep-

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arated from the popliteal space by the tibial attachment of the soleus muscle. The bones were not invaded. Photographs of the leg before amputation and of the cut surface of the tumor can be seen in the paper [1] by one of us (A. P. S.) already referred to.

After explantation of sterile neoplastic tissue, portions were fixed in Helly's and Bouin's fluids and in 25 per cent choral hydrate. Hematoxylin and eosin; Masson's aniline blue, acid fuchsin, ponceau trichrome stain; phosphotungstic acid hematoxylin; Laidlaw's silver carbonate reticulin stain and Cajal's silver impregnation were used.

Only after long study with the stains mentioned was it possible to interpret the morphological characteristics of this tumor. It consisted basically of an enormous number of capillary vessels, some of which contained red blood cells, but the majority of which did not. These tended to anastomose one with another and ran at haphazard in every direction. A few were lined by normal endothelia but the majority by larger spindle-shaped tumor cells. These had usually proliferated to such a degree that the vascular lumen was obscured and in ordinary stains one could see only cords or columns of cells running in various directions. It was only when such cords were found directly continuous with tubes containing red blood cells that one could suspect that they were obscured vessels (Fig. 2). This suspicion received confirmation from the silver reticulin stain which showed that the cell columns were enclosed within a capillary reticulin sheath. Between these tubes and solid cords were many other cells of a similar aspect supported by a matted, tangled framework of fine silver-blackened reticulin fibers.

Most of these tumor cells had a relatively mature aspect and mitoses were difficult to find. Their ovate nuclei contained two or more small nucleoli and were sharply defined. The cytoplasm was rather scanty, well defined, faintly acidophilic and slightly granular. At its edges the tumor cells infiltrated the surrounding muscles and aponeurotic tissues by sending out short tongue-like prolongations.

From this morphological study one gains the impression of vascular tubes in which there has been a marked overgrowth of swollen, spindle-shaped but otherwise mature endothelial tumor cells which have obscured the framework of the vascular tubes except when it is stained with silver carbonate and have also proliferated outside of the vascular tubes where they are associated with a rather dense meshwork of fine, tangled, reticulin fibers. One assumes that the endothelial cells have formed the reticulin fibers since no other source for them can be observed.

As already stated, this tumor differs in some respects from the other

17 hemangioendotheliomas available for study in this laboratory and some doubt was entertained regarding the interpretation of the findings upon a morphological basis alone. Fortunately, confirmatory evidence has been obtained from a study of the behavior of the explants.

Tissue Culture

Method

The medium used for cultivation consisted of one part chicken plasma, three parts human placental serum and one part extract of 9 to 11 day chick embryos in a buffered saline solution. The cultures were explanted in lying-drops of the medium on coverslips according to the Maximow method, and incubated at 37° C. They were washed in buffered saline three times a week, at which time the liquid components of the medium were renewed. The saline solution used was that devised by Simms and Sanders[2] in 1942. The cultures were maintained in their original situation on the flying coverslip, without transferal, for as long as possible during the 4-week period of cultivation; but it became necessary to transfer most of them at least once, because of plasma liquefaction which could not be controlled by patching.

During this time different explants were fixed, at intervals, in a 4 per cent solution of formaldehyde, and in Zenker's, Helly's and Bouin's fluids. They were stained with Delafield's hematoxylin, Mallory's phosphotungstic acid hematoxylin, Weigert's hematoxylin with mucicarmine, and fuchsin-ponceau-aniline blue, or impregnated with silver by McKinney's[3] modification (1929) of the Bielschowsky method, for reticulin.

Growth Characteristics

This tumor produced a sturdy, spiky growth within 24 hours. At 3 days the outgrowth had become flat and semimembranous, the cells tending to lie closely together, but not always appearing contiguous. A good many wandering cells of the macrophage type were present. At the outer boundary of the advancing sheet the cells were characterized by many needle-shaped pseudopodia pointing in a centrifugal direction (Fig. 8). The area of growth became an area of plasma liquefaction which terminated abruptly just behind this peripheral rank of cells with aiguilliform prolongations. Almost without exception the outgrowth advanced over the lower surface of the plasma clot, not through it, and lay flat against the coverslip like a membrane. Near the explant the cells tended to be elongated and slender, but toward the periphery where there was less crowding they became flatter and wider.

The outer boundaries of the cytoplasm were hardly to be discerned in the living state; consequently what appeared then, and after use of the common fixatives and stains, to be interstices between the cells may often have been areas of attenuated ectoplasm; for silver nitrate impregnation demonstrated a clear mosaic formed by the blackened cement borders of the cells (Fig. 7). After such fixatives as Bouin's, Helly's, or Zenker's fluids, all of which produce some shrinkage, there often seem to be gaps between the cells (Figs. 4 and 6). This may be an artifact of fixation, or it may be that these gaps are actually bridged by an ectoplasm too thin to be visible with the ordinary stains, as is often the case in mesothelial membranes and in capillary endothelium. The membranes formed by this tumor, however, are readily distinguishable from mesothelial membranes as we have seen them in cultures of normal and neoplastic serosae (Stout and Murray,[4] 1942). These latter are more compact and more regular as to cell boundary. In some areas the tumor cells adopted a more fibroblast-like form, taking up positions at a more or less uniform distance from one another. The distance was in some cases so great that it was difficult to believe that these cells were contiguous.

Frequently the long axes of the cells forming a single group were oriented all in one direction. Where the outgrowth was composed of several groups, this produced the effect of streams of cells meeting, crossing, or becoming confluent (Fig. 4). Such orientation phenomena were most likely due to convection currents in the medium, which changed direction during the course of the cultivation handling (Weiss,[5] 1934). It is interesting that such diverging and converging streams of cells simulate the conditions indicated by sections to exist in this as in many other tumors.

The cells growing out from this tumor were unusually large. (*Cf.* culture of fetal rat vascular endothelium photographed at same magnification, Fig. 5.) The nuclei were round to oval, containing one to four nucleoli, one and two being the most common numbers. Binucleate and sometimes multinucleate cells were seen. In the cytoplasm close to the nucleus, and often surrounding it, there were usually to be found small, uniform, moderately refractive granules which stained red supravitally with neutral red. If the cultures were rinsed with Maximow's lithium carmine, after 2 or 3 days carmine inclusions could be seen in the cells, usually occupying a perinuclear position.

Silver staining for reticulin after a fortnight or more of cultivation demonstrated a fine but very distinct network of fibers emerging from

the explant and tapering to nothing at about half the radius of the outgrowth (Fig. 1).

Discussion

Assuming that this is a primary growth in the leg, the majority of the histological possibilities for a tumor at this site could be eliminated by recourse to the sections, leaving the omnibus fibrosarcoma and neoplasms of the musculature or lining of the blood vessels as possible sources. A study of the growth characteristics of the tumor *in vitro* disposed rather readily of the first two alternatives and rather consistently supported the view arrived at independently by a consideration of the sections along with a series of other hemangioendotheliomas.

This generally membranous habit of growth, covering surfaces and forming a mosaic with cemented cell borders, does not characterize fibrosarcomas, nor any form of smooth muscle, normal or neoplastic, which is known to us. It cannot be called *typical* of vascular endothelium, but has been seen by us and reported by others in this connection. Most typically, vascular endothelium seems to grow *in vitro* in the form of solid or hollow tubes, as described, *e.g.*, by Lewis,[6] 1931, and Scriba,[7] 1935, in material from the embryonic chick. But Lewis[8] also reported (1922) an epithelioid type of outgrowth (rather similar to ours) from the liver sinusoids. Others, as Silberberg,[9] 1929, and Bisceglie,[10] 1930, described outgrowing endothelial cells as taking on a form indistinguishable from that of the fibrocyte. This has sometimes occurred in our cultures and we have been inclined to regard it as one of the responses to cutting and transfer, a process which habitually interferes with differentiation in the cut region.

In cultures of a hemangioma (unpublished) we have observed both the fibrocyte outgrowth, which may have originated from connective tissue involved in the tumor, and a sheet-like proliferation composed of cells similar to those which characterize this hemangioendothelioma. And in fetal rat cultures we have observed an interesting combination of the tubular type and membranous type of endothelial habit. This occurred in explants from ribs of 20 days' gestation, at which time ossification and vascularization are proceeding *pari passu* in these bones. Figure 3 shows a network of capillaries at one end of the rib and a flat membranous outgrowth from the other. In the former the transition takes place peripherad from tubular to flat type, and in the latter, from membranous to tubular. The presence of both sinusoid and capillary endothelium in a normally developing bone makes this observation of some interest.

Under normal conditions *in vivo* or *in vitro* vascular endothelium does not stain supravitally nor phagocytize particulate matter, as the tumor cells of our cultures did. But according to McJunkin,[11] 1927, the endothelium of liver sinusoids (of the adult rabbit), when properly stimulated, will do both. The sinusoids are regarded generally as a primitive type of capillary. It is not improbable that neoplastic vascular endothelium should behave similarly in some respects to embryonic or stimulated sinusoid endothelium.

Cameron and Chambers[12] reported the cultivation of a "congenital angioma . . . diagnosed as angio-endothelioma," which produced *in vitro* a variety of cells, and was characterized by the formation of "endothelial tubes." This appears to belong to a different group of vascular neoplasms from that with which we are concerned. Tubular structures which first appeared as solid cords composed of "endothelial cells" were described by Coman [13] as developing in tissue cultures of a human "angiosarcoma" which had metastasized to a rib. Other than this no histological details were given. Consequently it is difficult to classify this tumor with exactitude.

Summary

A hemangioendothelioma of unusual morphological appearance is described and its behavior *in vitro* is discussed. This particular neoplasm is regarded as manifesting itself in the form of a primitive or somewhat dedifferentiated vascular endothelium.

We are greatly indebted to Mrs. Irene A. Pogogeff for technical assistance in tissue culture and histology.

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[*Illustrations follow*]

DESCRIPTION OF PLATES

PLATE 49

FIG. 1. Reticulin fibers formed in the midzone of tumor growth. Seventeen days *in vitro*. Formaldehyde fixation, McKinney-Foot-Bielschowsky silver impregnation. × 310.

FIG. 2. Detail photomicrograph of a section of the tumor showing a vascular space containing red blood cells lined by the spindle-shaped tumor cells. Vaguely defined cords of tumor cells pass outward from it. No normal endothelia are present. × 480.

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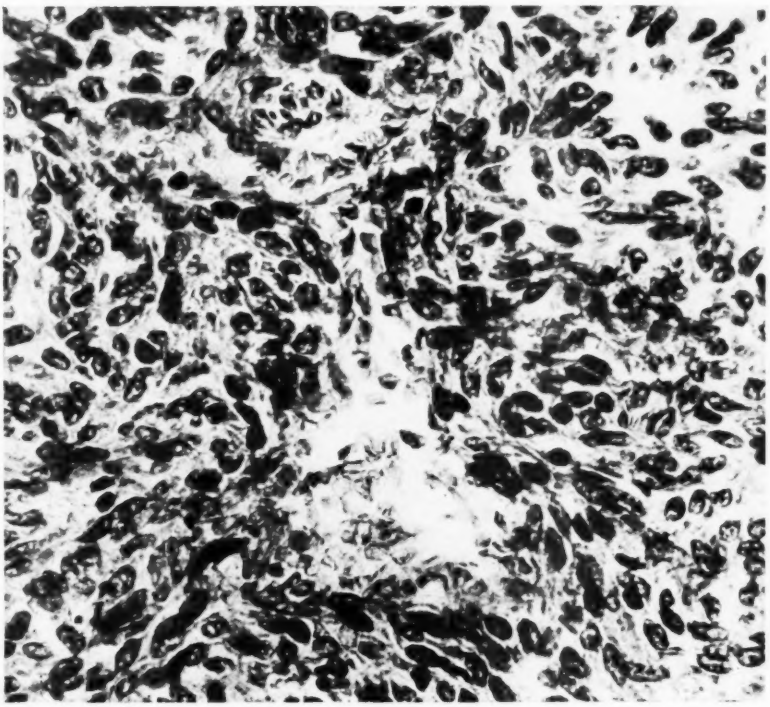
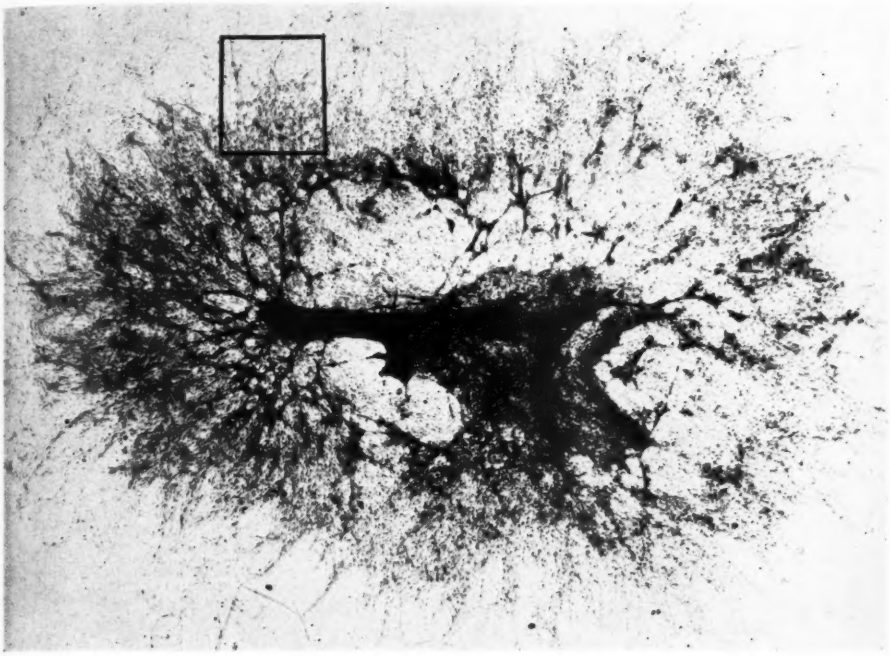


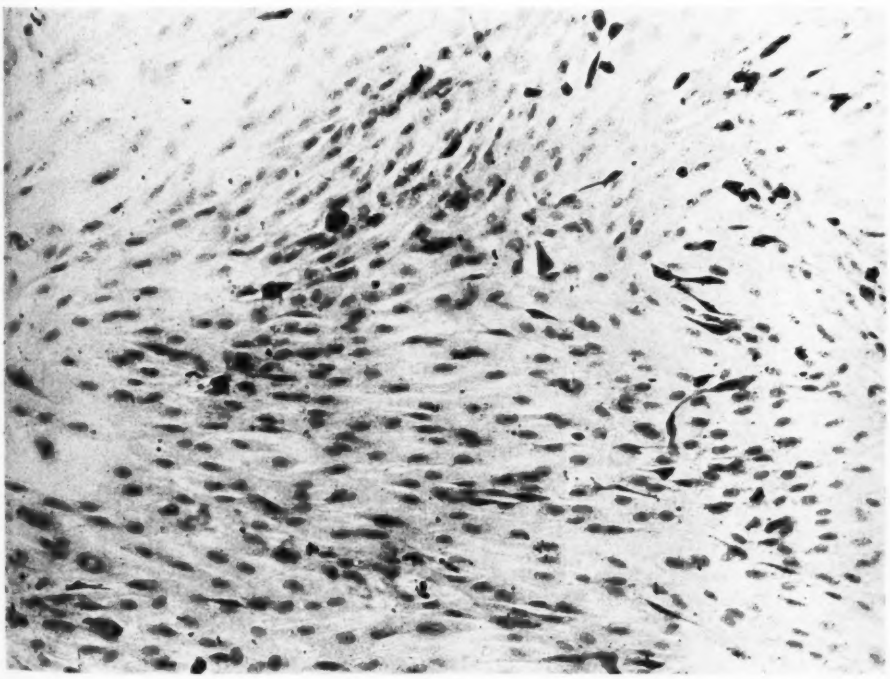
Murray and Stout Cultural Characteristics of Hemangioendothelioma

PLATE 50

FIG. 3. Six-day culture of a rib from a 20-day rat fetus, showing endothelium in capillary formation at left and in membranous habit at right. Towards the periphery the capillary cells spread out in sheet-like form. Helly's fixation, Delafield's hematoxylin stain. × 28.

FIG. 4. Seventeen-day culture from the tumor, showing a stream-like pattern of cell growth. Macrophages may be seen. Bouin's fixation. Delafield's hematoxylin stain. × 125.





Murray and Stout Cultural Characteristics of Hemangioendothelioma

PLATE 51

FIG. 5. Detail of peripheral sheet-like endothelial growth from the rib of the fetal rat shown in Figure 3. × 255.

FIG. 6. Detail of sheet-like growth from a culture of the tumor; 14 days *in vitro;* Helly's fixation, Delafield's hematoxylin stain. × 255.

FIG. 7. Similar growth from the tumor stained with silver nitrate to show mosaic of cemented cell borders. Fourteen days *in vitro*. × 255.

FIG. 8. Cell at periphery of 10-day culture, showing needle-shaped pseudopodia. Zenker's fixation, phosphotungstic acid hematoxylin stain. × 255.

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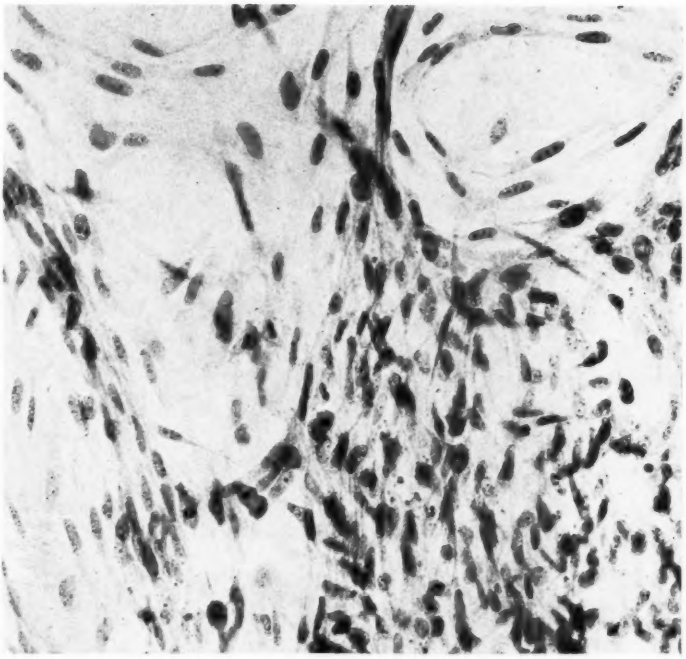

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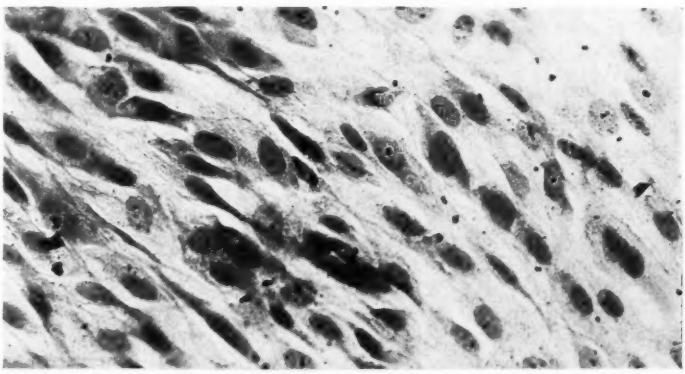

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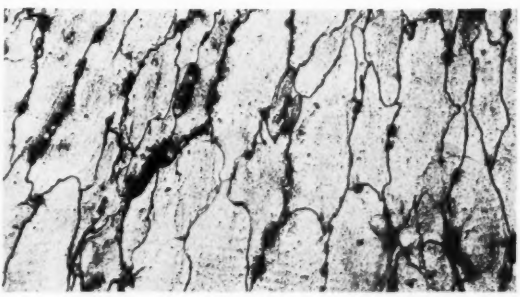

7

STUDIES ON THE DECALCIFICATION OF BONE*

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The problem of combining rapid and satisfactory decalcification and satisfactory Romanovsky staining of bone marrow remains one of difficulty. Some years back a series of experiments was made which led to our adoption of 5% formic acid in water as a decalcifying agent. This has proved quite satisfactory in routine use since 1936.

In the original study a survey was made of the effect of immersion for varying periods of time in various decalcifying agents on Romanovsky staining of rabbit marrow removed from the long bones. Periods as short as 1 day in 5% HNO_3 and 1 to 2 days in 5% SO_2 often seriously impaired marrow staining with eosin azure stains, producing a diffuse oxyphilia of nuclei as well as of cytoplasm. Treatment of marrow for 1, 2, or 3 days with our then routine decalcifying fluid: 25 cc. concentrated formic acid, 10 gm. sodium citrate, 75 cc. water; with saturated H_2SO_3 (5 to 6% SO_2), with citric acid and disodium phosphate buffer mixtures of pH 2.2, 3.0, 4.0, 5.0 and 6.0; with 6.35% NH_4NO_3 (the equivalent of 5% HNO_3), and with 14.65 NH_4Cl (the equivalent of 10% HCl) permitted excellent marrow staining after subsequent imbedding and sectioning.

Believing that injury to marrow staining might be controlled by regulation of the initial pH of the fluid, series of buffered mixtures of sulfurous and formic acids were made and pieces of rabbit bone marrow without bone were soaked in them for varying intervals, then washed, dehydrated, imbedded in paraffin, sectioned and stained by a Romanovsky method. With the H_2SO_3-sodium citrate series, 7 days' soaking in mixtures of pH 2.75 to 5.0 still permitted excellent staining, while even 1 day at pH 1.15 to 1.30 destroyed the basophilia of nuclear chromatin. Intermediate pH levels gave intermediate effects, varying with the pH level. In this series pH levels had to be determined electrometrically. With formic acid-sodium citrate mixtures ranging from pH 1.8 to 3.15, tissues soaked for 1 week at pH 3.0 or higher still stained well, while at pH 2.0 or lower for 2 to 4 days staining was poor. At 37° C. these mixtures produced much more rapid deterioration of staining quality.

These preliminary studies indicated that a decalcifying agent which

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did not produce a pH level below about 2.0 and did not take over 4 days would be desirable.

This led us to try various buffered mixtures of formic, sulfurous and trichloracetic acids as well as other substances for the decalcification of monkey bones fixed with dilute formaldehyde or with Orth's fluid. Among these were the ammonium salts which are known to dissolve appreciable quantities of calcium carbonate. On trial, 6.35% NH_4NO_3 solution, equivalent to 5% HNO_3, actually dissolved 68 mg. $CaCO_3$ per 100 cc. in 24 hours, and 14.65% NH_4Cl, equivalent to 10% HCl, dissolved 107 mg. $CaCO_3$ per 100 cc. in 1 day. (The analyses were made by Senior Chemist Elias Elvove.) These two solutions partially decalcified blocks of monkey vertebra in 13 and 17 days, respectively, yielding poor sections with much remaining demonstrable calcium in bone and excellent Romanovsky staining. Both solutions had an initial pH of 5.1 and might be of value for slow decalcification of cancellous bone where avoidance of acid was important. Monkey femora were still hard after 3 weeks.

White [1] used a neutral solution of ammonium citrate (pH 6.0 to 8.0), equivalent to about 6% citric acid. He said nothing about the time required.

Similarly, 5% KH_2PO_4 with an initial pH about 5.0, gave decalcification of decorticated cancellous bone from femur and sternum in 2 to 3 days. Sections were good, hematoxylin stains did not indicate any gross amount of remaining calcium and Romanovsky staining was excellent. However, femoral shaft, knee joints and vertebrae were still hard after 18 days.

The 10% sodium citrate solution in 25% formic acid (which we were then using routinely and which is still widely used, though I do not know its source) has an initial pH of 2.35 and decalcifies monkey femora in 2 to 3 days, vertebrae in 24 to 36 hours and decorticated cancellous bone in 24 hours. Prolonging the exposure to 96 hours still permitted excellent marrow staining. Evans and Krajian [2] used a similar mixture of equal parts of 85% formic acid, 95% alcohol and 20% sodium citrate solution. This decalcified in 3 to 4 days and allowed good staining.

A mixture containing equal weights (27.5 gm.) of formic acid and sodium citrate, with an initial pH of 3.15, decalcified monkey vertebrae in 4 days and femora in 8 to 9 days. Subsequent marrow staining was good, and remained so with vertebrae allowed to remain for 4 additional days in the fluid.

Disodium and trisodium phosphate-formic acid mixtures with initial

pH levels of 1.8 to 2.65 decalcified monkey vertebrae in 1 to 2 days and femora in 1 to 12 days, the interval increasing with rising pH level, the quality of the sections decreasing conversely and the brilliance of Romanovsky staining satisfactory in all, but best at the highest pH level of decalcification.

Solutions containing 1 to 3% by volume of 90% formic acid (1.1 to 3.3% by weight of pure formic acid) (pH 1.9 to 2.1) decalcified rather slowly, with good subsequent marrow staining and fair cutting consistency. Those containing 4 to 25% unbuffered formic acid (pH 1.8 to 1.4) decalcified monkey vertebrae in 1 day, femora in 1½ to 3 days, with best results in about 2 days. Higher concentrations appeared to be no better than 5%, and this level gave results equal to any of the better buffered mixtures.

Similar unbuffered 1 to 25% aqueous dilutions of acetic acid (pH 2.8 to 1.9) took 2 to 4 days to decalcify monkey vertebrae, 10 to 12 days for femora, and even then sections were only fair and much calcium remained in the femoral cortex with the lower levels of acetic acid. Consistent with previous results with high pH levels in decalcification, Romanovsky staining of marrow was good with 1 to 10% acetic acid, poorer with 15 and 25% (pH 2.0 and 1.9).

Buffered mixtures of 25% acetic acid with 10 to 25% sodium citrate, with 2 to 8.3% Na_2HPO_4 and with 8 to 24% Na_3PO_4, with pH levels ranging from 3.4 to 4.6, generally failed to soften monkey femora in 3 weeks, and only partially decalcified vertebrae in 6 to 14 days, yielding fair sections, some of which showed demonstrable lime salts, and quite variable staining.

Solutions of 80% alcohol containing 25% formic acid, 25% acetic acid or 10 or 20% salicylic acid gave no evident softening of monkey bones in 3 weeks. Similarly, Carnoy fluids containing 60 parts absolute alcohol, 30 parts chloroform and 10 parts of either 90% formic or glacial acetic acid failed to decalcify during the fixation interval.

Saturated solutions of sulfur dioxide in water (5 to 6%) decalcified decorticated cancellous bone in 7 hours, monkey vertebrae in about 15 hours and femoral shafts in about 36 hours. Sectioning was generally good and subsequent staining satisfactory. With longer exposures Romanovsky staining of marrow was impaired.

In 5% HNO_3 monkey femora, vertebrae, sterna and other bones were decalcified in 24 to 36 hours. Sections were fair to good, iron hematoxylin-picrofuchsin stains were uniformly satisfactory and Romanovsky staining gave diffuse nuclear and cytoplasmic oxyphilia.

Similarly, trial of Wilson's [3] rapid method using 20% HNO_3 *in vacuo*,

gave diffuse oxyphilia of all structures except cartilage, mucus and bone cells after only 5 hours' decalcification. Shorter intervals such as the ½ to 3 hours claimed by Wilson for this method failed to decalcify half skulls of rats. Substitution of 25% formic acid in this procedure increased the decalcification time to 8 to 24 hours, but this time permits excellent Romanovsky staining of the surrounding soft structures as well as bone and marrow.

Since decalcification of similar bones may often be accomplished in 24 hours with 25% formic acid, it is questionable whether Wilson's[3] preliminary defatting procedure has any great influence. Comparative tests made by immersing similar bones in the same formic acid solution *in vacuo* and in air showed no difference in the length of time required for decalcification.

DeGalantha's[4] complicated HNO_3, alcohol, picric acid, olive oil formula claims no quicker or better decalcification than we have obtained with 5% formic acid, and his statement about subsequent staining does not cover the sensitive Romanovsky methods.

Monkey vertebrae and femora were decalcified in 24 hours in 5% trichloracetic acid, sections were good and subsequent iron hematoxylin-picrofuchsin and Romanovsky stains were satisfactory. However, on account of the high molecular weight (163) a larger amount is required to avoid exhaustion than with formic acid (mol. wt. 46) or sulfurous acid (½ mol. wt. = 41), and the present (1943) cost of trichloracetic acid is four times that of formic acid.

Since 5% formic acid was as efficient a decalcifying agent as any of those tried which did not also seriously impair Romanovsky staining of bone marrow in a short time, two series of decalcification of weighed samples of shaft of monkey femur and tibia were made with varying proportions of 5% formic acid, to determine the necessary quantity for prompt decalcification. In the first series bones were removed as soon as apparently decalcified, in about 2 days, washed and weighed, then dehydrated, sectioned in paraffin and stained with iron hematoxylin-picrofuchsin and with the buffered Romanovsky stain. In the second series the blocks were left in the same decalcifying fluid for twice the time necessary for apparent decalcification, then washed, weighed and treated as before. Blocks treated for 2 days with 10 to 30 cc. of 5% formic acid per gram lost an average of 25% of their weight; those treated with 40 to 100 cc. lost 33%. Those treated for 4 days with 10 to 30 cc. per gram lost 31%; those with 40 to 100 cc., 32%. All sectioned well and Romanovsky and van Gieson connective tissue stains were satisfactory.

Substitution of 5 cc. of 90% formic acid for the usual 5 cc. of glacial acetic acid in Zenker's fluid gives at least partial decalcification in 24 hours, much better even than the 10 cc. glacial acetic acid often recommended for this purpose, and the usual picture of Zenker's fixation seems little altered. Likewise, inclusion of 5% formic acid in Bouin's fluid in place of the usual acetic acid makes it a quite efficient decalcifying agent. Similarly the "PFF" fluid recently reported from this laboratory,[5] which contains 5% formic acid, and 10% strong Formalin (4% formaldehyde), saturated with picric acid, decalcifies small bones well in 1 to 2 days.

The fluid of McNamara, Murphy and Gore[6] ($HgCl_2$, 10; trichloracetic acid, 30; concentrated HNO_3, 5; strong Formalin, 40; 95% alcohol, 50; water, 400) is said to require 3 to 5 days for decalcification, and to impair nuclear staining in over 7 days. They do not note its effect on Romanovsky staining.

Conclusions

Slow decalcification of decorticated cancellous bone with excellent subsequent marrow staining by Romanovsky stains may be accomplished with ammonium nitrate, ammonium chloride, or potassium acid phosphate, but decalcification of cortical bone is too slow for practical use.

Buffered sodium citrate formic acid mixtures with initial pH above 3.0 are a little faster, decalcify cortical bone slowly and permit good marrow staining even with several days' exposure beyond apparent decalcification.

Buffered formic acid solutions with initial pH around 2.5 are faster, but show more tendency to impair marrow staining and are generally no better in either respect than 5% aqueous formic acid, which apparently is as good as higher concentrations of the same acid.

Acetic acid is a relatively inefficient decalcifying agent, and appears to show somewhat less margin of safety between adequate decalcification and impairment of chromatin staining.

Trichloracetic acid is a good decalcifying agent, but more is required, and the cost is higher than for formic acid.

Nitric and sulfurous acids are prompt decalcifying agents, but the first as promptly spoils Romanovsky staining of marrow, and the second tends to do so if decalcification takes 2 days or more.

With 5% formic acid, 40 cc. per gm. of bone should be used for prompt decalcification.

For simultaneous fixation and decalcification, addition of 5 per cent

formic acid in place of acetic can be recommended in such fluids as Zenker's, Bouin's and "PFF."

Eighty per cent alcohol solutions of formic, acetic and salicylic acids do not decalcify, nor do acetic or formic Carnoy's fluids.

The use of vacuum during decalcification is of no particular advantage except to remove bubbles in large specimens, and preliminary defatting apparently saves little time in the decalcifying fluid.

I have referred freely to the various manuals on histologic technic (Mallory's,[7] Lee's,[8] Schmorl's,[9] Romeis',[10] Langeron's [11]) but have attempted no complete review of the literature.

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TOOTH BUDS AND JAWS IN PATIENTS WITH CONGENITAL SYPHILIS

Correlation between Distribution of Treponema pallidum and Tissue Reaction *

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Introduction

Shortly after the discovery of *Treponema pallidum* many attempts were made to demonstrate it in the bones of stillborn and living-born congenitally syphilitic infants. However, these early efforts failed due to the imperfect histologic methods of that time. It was not until Levaditi[1] (1905) and Bertarelli and Volpino[2] (1906) introduced their staining methods that *T. pallidum* was occasionally demonstrated in sections of a single bone of congenitally syphilitic fetuses or infants. The first, and as yet not repeated, systematic study on the distribution of *T. pallidum* in all bones of congenitally syphilitic stillborn and living-born infants was conducted by Schneider.[3] He must be given credit for having proved definitely that the various bone changes in congenital syphilis, such as osteochondritis, periostitis and osteomyelitis, are but localized tissue reactions to the invasion of *T. pallidum* and to the toxins of broken-down spirochetes.† Schneider's study showed that bone is the favorite site for the spirochetes, which lodge and multiply tremendously, particularly in those parts of the bone wherein very active growth is going on. The reaction to the appearance of the organism in these areas of bone is a very definite inflammation of the osteogenetic tissue resulting in an interference with normal osteoblastic action.

With the spread of *T. pallidum* throughout the whole skeleton in congenital syphilis, the organism was expected to be found in the jaws and also within the tooth buds. However, the lack of adequate material and the difficulty of staining spirochetes in bone sections led to the advancement of the theory that the enamel lesions of the well known Hutchinson's teeth result from a syphilitic disturbance of endocrine glands, specifically the parathyroid glands. This theory, brought forward particularly by Kranz,[4] found as many supporters as the theory that a superimposed rickets is the cause of Hutchinson's teeth. These concepts were not abandoned or were not even considered doubtful

* Received for publication, June 14, 1943.
† Throughout this article "spirochete" refers to *Treponema pallidum*.

when Cavallaro,[5] Pasini[6] and Del Guasta[7] occasionally succeeded in showing spirochetes only in the vessel walls of some tooth buds. These authors limited their studies to the proof of the presence of *T. pallidum* in a small area, for instance in the pulp, but did not deal with the tissue changes and certainly not with the correlation of tissue changes with spirochetosis.

In this country the first attempt to demonstrate *T. pallidum* in the tooth buds of syphilitic fetuses and to correlate the reaction of the tissue with the Hutchinson's deformities was made by Hill.[8] Hill was correct in stating that the "hypoplasia of the enamel cannot be explained as the result of spirochetosis unless the organisms can be found intimately associated with the areas of degeneration." However, he was not able to prove the presence of spirochetes in the "dental anlage" of nine suspected congenital syphilitic fetuses using the staining technics of Levaditi, Warthin-Starry, Jahnel and Giemsa. Therefore, Hill concluded "that there is insufficient evidence to justify the conclusion that the characteristic congenital syphilitic dental deformities are the direct result of the invasion of the enamel organ by the *Treponema pallidum*."

Simultaneously with Hill's [8] article, I [9] published in 1931 the results of microscopic studies of four congenitally syphilitic fetuses and infants. I demonstrated not only the presence of innumerable *T. pallidum* in the pulp, the dentinoid, the dentinal tubules, the tooth sac, the ameloblast layer and even in the stellate reticulum, but I was also able to demonstrate the reaction of the tissue to the spirochetosis by dividing the jaws in midline, using one-half for the spirochete demonstration while the other half served for tissue studies. The correlation between the presence of innumerable spirochetes and the inflammatory reaction on the part of the tissue, resulting in degenerative changes of the ameloblasts and odontoblasts, became evident. The tooth buds so involved were those of both the deciduous and the permanent teeth.

My conclusions that the characteristic lesions of the Hutchinson's teeth are due to the syphilitic inflammation within and around the tooth buds were fully confirmed by Pflüger,[10] who studied the jaws of nine syphilitic infants microscopically but did not attempt to demonstrate the organism in the tissue.

Boyle,[11] in his histologic findings in the tooth buds of syphilitic fetuses, stressed the presence of inflammatory changes and their results. However, he particularly emphasized the defective calcification of the dentin as represented by numerous interglobular spaces and extremely

wide zones of predentin. Boyle made no reference to the presence of the spirochetes.

Burket [12] examined the jaws of two congenitally syphilitic infants, one 4 and the other 8½ months of age, both of whom received antisyphilitic treatment. He was unable to show the organism in the dental tissue. Nevertheless, he concluded that the localized changes which he described, "such as the enamel hypoplasias, metaplasia or aplasia of the ameloblasts, could conceivably be produced by the local activity of the *Treponema pallidum*." According to Burket, the changes of the buds of the permanent teeth were confined to the enamel organ while those of the buds of the deciduous teeth were evident only in the dentin and pulp.

Since the authors of even the recent editions of textbooks of oral pathology have been reluctant to accept my conclusions as to the pathogenesis of Hutchinson's teeth and are inclined to adopt the endocrine origin, I have made further studies on the jaws of six congenitally syphilitic but nonmacerated subjects: four fetuses of 6, 8, and 9 months and two infants of 2½ weeks and 1½ months of age.

Two of the fetuses exhibited radiographically and microscopically an osteochondritis syphilitica and periostitis syphilitica (Fig. 1), while the other two fetuses and the infants revealed only a periostitis syphilitica of the long bones and the jaws.

This sequel to my previous investigations is concerned with the following main questions:

1. Can *T. pallidum* be demonstrated in the tooth buds and jaws of congenitally syphilitic fetuses and infants?

2. Can the lesions of these organs in congenital syphilis be related to an endocrine disturbance of calcium and phosphorus metabolism?

3. Are the tooth buds of both dentitions involved and does the involvement always evolve symmetrical lesions?

Staining Method

The jaws were divided in the midline and most of the long bones and some ribs and the claviculae were cut in half in their long axis, thus providing equal parts of the same bone for the spirochete stain and for the routine hematoxylin and eosin stain.

The silver impregnation after Bertarelli and Volpino,[2] a modified Levaditi method, proved to be the technic of choice to demonstrate the *T. pallidum* in celloidin or paraffin sections of about 5 to 6 μ thickness. The specimens, carefully and thoroughly decalcified in 5 per cent nitric

acid, were divided into small blocks, neutralized, washed and placed in the silver solution at 37° C. for 18 days, according to the method which follows. Decalcification does not hamper the staining of the *T. pallidum*. Bertarelli and Volpino's technic offers the great advantage that the sections, in which the spirochetes stain black, can be counterstained by hematoxylin and eosin, thus enabling the investigator to study simultaneously the distribution of the organisms and the tissue reaction.

Method for demonstrating *T. pallidum* in bone:

Precautions

1. Absolute cleanliness.
2. Bottles should be dark amber.
3. Solutions must be prepared immediately before use.

Technic

1. Fix in a 2% solution of formaldehyde or in alcohol. Alcohol fixation requires a longer time.
2. Decalcify very thoroughly in large quantities of 5% nitric acid.
3. Cut the bones into blocks 5 to 10 mm. thick.
4. Place the blocks in 5% sodium sulphate for 2 days.
5. Wash in tap water for 2 days.
6. Place in the following solution, freshly prepared:
   Silver nitrate—1.5 gm.
   Water, distilled—50 cc.
   96% alcohol—50 cc.
   Glacial acetic acid—4 to 5 drops

This silver impregnation must be carried out in complete darkness at 37° C. for about 18 days. Change the solution when it becomes cloudy.

7. Wash in distilled water for 1 day, changing frequently.
8. Place in the following solution for 48 hours in darkness at room temperature:
   Tannic acid—3.0 gm.
   Gallic acid—5.0 gm.
   Sodium acetate—10.0 gm.
   Water, distilled—350 cc.

Change solution when it becomes cloudy.

9. Wash carefully in distilled water, changing several times a day.
10. Place successively in 70%, 80%, 95% and absolute alcohol.
11. Embed in paraffin or celloidin. If paraffin is used, interpose cedar-oil between xylol and paraffin.
12. Cut sections 5 μ in thickness. The treponemes are black; the tissue appears yellow. The sections may be counterstained with hematoxylin and eosin.

Distribution of T. pallidum in the Tooth Sac and the Enamel Epithelium

The structure of the spirochetes seen in the bone and tooth tissues varied from the typical long, spiral type to various degenerative forms. The organisms in most of the fetuses constantly appeared tightly twisted with pointed ends and with regular spirals of regular amplitude

and depth. The organisms in stillborn or livingborn infants, however, occasionally showed regressive changes as to size and shape. Their ends were thickened and rounded, the number of the spirals markedly reduced; some were partially straight, their bodies appearing granular and finally breaking into many pieces. The older the infant the fewer were the spirochetes. The results of my study of the distribution of the *T. pallidum* in the skeleton showed that the tooth sacs of the tooth buds of the deciduous teeth and those of the permanent teeth harbor a greater amount of organisms than any other tissue of the osseous system. Moreover, even if the spirochetosis gradually disappeared, as was seen mostly in syphilitic infants, and if the periosteum and even the primary bone marrow next to the epiphysis contained scarcely any organisms, there were masses of them demonstrable in the tooth sac of the buds of the deciduous and permanent teeth.

This fact might be explained as being in accordance with Schneider's[3] observation of a striking accumulation and persistence of *T. pallidum* in bony areas of pronounced growth activity, which I was able to confirm. They were more persistent there than in the pulp. It is obvious that the extreme vascularity of the tooth sac tissue adjacent to the enamel epithelium and the Hertwig's sheath played an important rôle in the spread of the organisms. It is the blood stream that carried them into the tissue. The organisms penetrated through the blood vessel walls and moved into the tissue where they multiplied. There were innumerable spirochetes in the tooth sac layer next to the enamel epithelium (Fig. 2). They formed braid-like masses of twisted organisms which were brought there by the dense network of capillaries that intruded the outer epithelial layer (Fig. 3). It did not make any difference whether the outer epithelial layer still outlined the stellate reticulum or already covered the stratum intermedium as a constituent of the united epithelium.

While the spirochetes lay interspersed between the fibrous tissue of the alveolar and intermedial layer of the tooth sac, arranged in stripes, they formed dense whorls within the destroyed enamel epithelium. Yet relatively few of them penetrated into the ameloblast layer between the cells. Here they rested parallel to the ameloblasts, while others, having advanced farther, lay between the ameloblasts and the enamel surface and parallel to the latter. A similar spread occurred in the region of the Hertwig's sheath where numerous organisms were observed both outside and inside this epithelial loop. The organisms were noticed also in the tooth sac of the tooth buds of the first permanent molars.

The stellate reticulum, fully preserved, contained only a very few

spirochetes scattered here and there, which might be due either to its avascularity or to the protection by the outer epithelial layer. Indeed, the continuous enamel epithelium seems to be a barrier against the invasion of *T. pallidum* and must be destroyed at least partially to pave the way for their advance.

Distribution of T. pallidum in Pulp and Dentin

On the whole the organisms were less numerous in the pulps of the tooth buds of the deciduous teeth and the first permanent molars than in the dental sacs. They were observed in the basal part rather than in the coronal area of the pulp, and there particularly in the region around and between the odontoblasts. They were transmitted to this area by the dense loops forming the capillaries of the pulp. While quite a few spirochetes appeared embedded in the predentin, some of them penetrated further into dentinal tubules. This is possible, since the thickness of the dentinal tubules varies from 1.3 μ to 4.5 μ while the average thickness of *T. pallidum* does not exceed 0.25 μ (Fig. 4).

Wherever Hertwig's sheath intervened between pulp and tooth sac the organisms in the pulp tissue were less abundant than in the adjacent sac. Here again one leans to the idea that it might have been this epithelial layer that prevented the organisms lying in the tooth sac from invading the pulp through the intact Hertwig's sheath.

Distribution of T. pallidum in the Jaw

The distribution of organisms throughout the jaws was not uniform. There was a striking difference between the abundant spirochetes in the tooth sac and the relatively small amount of organisms in the bone. While the bone marrow next to the tooth buds still contained a fair number of spirochetes, they decreased rapidly toward the outer areas but increased again in the new periosteal bone layer and in the periosteum. However, nowhere within these bones did the organisms appear in such dense masses as within the tooth bud. Many of them showed degeneration such as shortening and breaking up into small fragments or even into granules, whereas those around and within the tooth bud were very well preserved.

The spirochetes in the bone marrow were mostly concentrated around the capillaries and occasionally around the osteoblasts. Such osteoblasts, together with the organisms, had been included within the bone substance. This may explain the finding of bone cells, the lacunae of which were filled with spirochetes. Of course, such osteocytes notably were observed within the newly formed bone trabeculae of

periosteal and endosteal origin, in areas of extensive inflammatory reaction (Fig. 5). Schneider,[3] who was the first to describe *T. pallidum* in osteocytes, advanced the idea that these organisms might be freed in the course of bone resorption later on, thus making a recurrence possible. Particles of broken-down spirochetes were found within leukocytes in the bone marrow. The periosteum of the jaws contained abundant organisms, mainly perivascularly arranged and better preserved than those in the bone marrow.

Tissue Changes

In the cases of congenital syphilis which I studied, I noted that the intensity of the tissue changes increased proportionally with the degree of degeneration of the spirochetes. Areas containing abundant, well preserved organisms showed minor tissue alteration. This observation agrees with the concept of Schneider,[3] who developed the idea from his studies that it is particularly the breaking down of the spirochetes that produces the tissue reaction. Of course, a different conclusion might be drawn from this observation: it might be the case that the inflammatory reaction gradually destroyed the spirochetes.

The tissue reaction varied with the age of the child: syphilitic fetuses showed less reaction than syphilitic infants. This might be explained by the still moderate capability of the fetal tissue to produce reactive changes to the invasion of the organisms, except in areas that harbor an extreme number of them. The tissue of an infant, however, more readily responds with inflammatory changes to the same irritation.

Changes of the Tooth Sac and the Epithelial Tissue of the Tooth Buds

The authors who studied the congenital syphilitic changes of the tooth buds overlooked the reaction of the tooth sac tissue, though it deserves the greatest attention. It is the mesenchymal structure that first and to the most striking degree undergoes changes due to the toxic effect of *T. pallidum*. The starting point of this involvement was the inflammatory reaction around the small, most frequently dilated, but sometimes obliterated vessels (obliterative endarteritis). The walls and the tissue about these vessels contained abundant spirochetes as was previously described. This cellular infiltration was composed of relatively few leukocytes, chiefly lymphocytes, and a startling number of plasma cells (Fig. 6). Dense layers of wavy fibrous tissue, interspersed with masses of collagen, were accumulated around the vessels. Extremely conspicuous strands of collagen, intensively stained with eosin, contrasted with the plasma cells and the other cells of chronic

inflammation which were scattered over the whole tooth sac. These changes occurred mainly in the layers of the tooth sac adjoining the tooth bud and the bony crypt. The islands of collagen adjacent to the bony crypt formed the matrix of atypical and primitive bone formation which was either in connection with the trabeculae proper of the bony crypt or free in the tooth sac (Fig. 7). Deeply blue-stained, irregularly calcified strands formed a latticework within the collagen, thus encircling and gradually replacing it. Connective tissue cells were embedded within these strands. This abortive bone was not produced by osteoblasts but was formed by infiltration of calcium salts into collagen and the surrounding connective tissue.

There were occasional large areas of exudate scattered throughout the fibrous tissue. They mainly occupied the region next to the tooth buds (Fig. 8).

All of the constituents of the enamel organ revealed changes. The tooth buds of the deciduous teeth were found to be more affected than those of the permanent teeth. The enamel epithelium showed alterations ranging from hydropic degeneration to partial or complete destruction, according to the intensity of the reaction in the tooth sac. Occasionally a small-cell infiltration was observed in this layer. Large masses of exudate intervened between enamel and enamel epithelium, detaching and destroying it in some areas (Fig 9).

The stellate reticulum, in which only a few spirochetes were seen, sometimes manifested no changes at all although other tissues of the tooth bud were extremely altered. However, occasionally degeneration or a stunted development was observed. The long processes connecting the cells with each other appeared thicker, or the cells had lost their stellate appearance, or the stellate reticulum was replaced by a fibrous reticulum which contained only a few cells or none at all. Sometimes the whole stellate reticulum disintegrated and only remnants of it were left.

Even though the stratum intermedium appeared to be somewhat hyperplastic or normal, the ameloblasts manifested striking alterations. There was a decrease in size of the ameloblasts. They either took up the appearance of common squamous epithelial cells or their arrangement became disturbed and at the same time they became elongated and bent. Here and there the continuity of the ameloblastic layer was broken up into small islands and edematous connective tissue of the tooth sac became adjacent to the enamel (Fig. 9). The most prominent changes, however, were noted in the cytoplasm of the ameloblasts. They showed hydropic degeneration which led to swelling and rupture

of the granular cytoplasm. Occasionally, the ameloblastic layer became detached from the enamel by exudate.

Hertwig's epithelial sheath in some instances was also markedly altered by degeneration. Its cells were either converted into a mass of cell débris or its affected layers appeared separated by exudate containing remnants of epithelial cells.

As to the changes of the enamel, two types of involvement stood out: there were globular depositions of abortive enamel between the normal enamel and the more or less affected ameloblasts (Fig. 10); or, there was only a thin layer of normal enamel without any coat of ameloblasts. The first was the imperfect product of damaged ameloblasts while the latter feature was brought about by a more rapid destruction of the ameloblasts, so that enamel formation became stunted.

Changes in the Dentin and the Pulp

It is important to emphasize that no disturbances of calcification of the dentin were observed. The thickness of predentin did not exceed the normal limit and its border toward the calcified dentin was sharp and even. The predentin did not contain any vessel or cell inclusions.

Changes in the pulps and dental papillae were not nearly so pronounced as they were in the other connective tissue structures and were observed only in infants. The vessels were dilated and surrounded by a moderate small-cell infiltration. A few hemorrhages were scattered through the pulps, the tissue of which was converted into fibrous tissue with pulp cells that had lost their protoplasmic processes.

Generally, the alterations of the odontoblasts were not such as would have been expected. These cells seemed to function quite satisfactorily despite the irregularity of their arrangement and the changes in their size. Occasionally, small areas of them had disappeared due to degeneration so that fibrous pulp tissue bordered the predentin.

There was one case, an infant, 2½ weeks old, with a very remarkable pulp involvement. The pulps of the upper central deciduous incisors were converted into granulation tissue extremely rich in round cells, interspersed with a few small, necrotic areas. No odontoblasts were found next to the exceedingly narrow predentin layer; however, there were fairly sharply circumscribed areas of dense small-cell infiltration, the plane contour of which was adjacent to the dentin while the convex contour expanded into the pulp and was surrounded by a fibrous layer (Fig. 11). Spotty areas of necrosis were found inside and outside these lesions which were located in the region of the rich vascular plexus about and between the odontoblasts. Although spirochetes

were not observed in these sections, probably because they were destroyed by the extreme tissue reaction, the tissue changes must be related to congenital syphilis. I am inclined to believe that prior to the tissue reaction these areas of massed capillaries were surrounded by dense clumps of organisms which subsequently were destroyed by a dense infiltration of leukocytes.

These roundish granulomas resembled the structures that were described as "miliary syphilomata" and were found by Herxheimer [13] in the liver, adrenals, lungs, spleen and bones of patients with congenital syphilis. The "miliary syphiloma," a term that is not an appropriate one, must not be confused with the specific gumma.

Changes in the Jaw

The bone marrow was converted into a fibrous-edematous tissue containing dilated vessels surrounded by an inflammatory infiltration consisting largely of plasma cells. Some vessels showed an endarteritis. The bone marrow of the peripheral parts of the bone consisted of a very dense fibrous tissue. Strands of collagen and cells were included in the calcification and formed the matrix of a sort of "bone" that developed without osteoblastic activity. This type of heavily calcified trabeculae was also laid down on the walls of the mandibular canal, thus encroaching upon it (Fig. 12). These calcified structures were abundantly developed and some of them formed the basis upon which normal bone was deposited by osteoblasts. Osteoclastic activity was almost negligible. Periosteal osteophytes, the product of syphilitic periostitis, made up a thick layer that consisted of the kind of "bone" previously described. They were in connection with the old bone or separated from it by granulation tissue rich in collagen and small-cell infiltrations.

The periosteum revealed chronic inflammation.

The Nature of the Lesions Described

Since the material of the present study was obtained from fetuses and infants of very early age, any thought that rickets might have produced the lesions must be excluded. Moreover, there was no microscopic evidence whatsoever of tissue changes that could be related to a systemic disturbance of calcium and phosphorus metabolism. While it is true that the changes of the ameloblasts and the results thereof resembled those seen in disturbances of calcium metabolism, the dentin and the bones in patients with congenital syphilis did not show any of the alterations that are so significant and peculiar to disturbances of calcium metabolism. There were no extremely wide zones of dentinoid or

osteoid, no irregular globular calcification of the dentin, no capillaries enclosed within the dentinoid such as I have described pertaining to human and experimental rickets and to any other severe illness that interferes with the normal calcification of the tooth bud and the bone. On the contrary, the calcification of the dentin and of the bones of the material studied must even be considered as excessive. However, more important is the fact that I have proved the presence of abundant spirochetes and of a reactive chronic perivascular inflammation composed mainly of plasma cells and lymphocytes, associated with a fibrosis and a production of collagen, the predominant feature of syphilitic inflammation.

The occurrence of periostitis or osteochondritis syphilitica, or both, in all of my cases—roentgenologically and microscopically observed—added further support to my concept that all changes in the jaws are syphilitic. No specific rachitic changes could be seen in sections of the long and flat bones. Furthermore, it is significant that the syphilitic lesions which I studied did not involve symmetrical groups of teeth of the same developmental period as is characteristic of systemic disturbances of calcium and phosphorus metabolism. The morphologic changes were noted also in single teeth.

In the alleged association of a systemic disturbance of calcium and phosphorus metabolism with the morphologic changes in congenital syphilis, one should expect changes in the parathyroid glands. However, Schneider [3] was unable to find alterations of these glands although spirochetes were very numerous in them.

The fact that congenital syphilis might be complicated with rickets or with some other disturbance of calcium-phosphorus metabolism must be admitted. In fact, scrutiny of the few cases employed by some authors for the study of the tooth bud changes in congenital syphilis convincingly showed alterations pathognomonic of rickets (in children above 3 months of age) or of disturbances of calcium metabolism of other origin (in fetuses or in younger children). Case no. 10 in Pflüger's [10] report belongs to this group and combines congenital syphilitic and rachitic lesions.

Boyle's [11] observation of a striking disturbance of dentinal calcification with broad predentin layers cannot be directly attributed to syphilitic action for the reasons mentioned.

Some of the cases microscopically examined showed syphilitic inflammation affecting tooth buds, of both the deciduous and the permanent dentition. It was due to the age of the cases studied that the lesions were more pronounced in the further developed buds of decidu-

ous teeth than in those of the first permanent molars, although there was no marked difference in the number of spirochetes about or within the organs of the two dentitions.

The inflammatory involvement of the odontoblasts and the ameloblasts of the buds of the first permanent molars was still slight because of their early developmental phase, yet there was a distortion of the shape of these buds. This disfiguration was brought about by the pressure of the extremely dense fibrous granulation tissue of the tooth sac. This observation explains fully the clinical conclusions of Sarnat, Schour, Shaw and Heupel [14] that "In congenital syphilis the teeth may show disturbances in the development phases which occur during the neonatal and earliest infancy periods. At this time the deciduous teeth are active in enamel formation (apposition) and may therefore show hypoplasia (chronologic enamel aplasia); while the permanent teeth which are active in morpho-differentiation may show a disturbed dentino-enamel junction with a resulting characteristic distortion of the crown (Hutchinson incisor, Moon molar)."

Summary

In disagreement with the results of some investigators, this study proved the presence of abundant spirochetes in the jaws and tooth buds of both dentitions in fetuses and infants with congenital syphilis. They were demonstrated in braid-like masses of twisted spirochetes in the highly vascular tooth sac, particularly next to the outer epithelial layer. They had migrated through the stratum intermedium into the ameloblastic layer and were also found in the avascular stellate reticulum. They were brought by the vessels into the dental papillae and pulps and then multiplied noticeably about the odontoblasts. They were seen in the predentin and within the dentinal tubules of the well calcified dentin.

The excessive number of spirochetes about and within the tooth buds must be attributed to the well known fact that the greater intensity of invasion of spirochetes is reached in areas of more intensive growth activity.

The nests of spirochetes within the osteocytes of bone trabeculae might help to explain syphilitic recrudescence. The bone marrow, and particularly the periosteum, harbored many organisms. In infants many spirochetes have undergone degenerative changes or have disintegrated.

The reaction of the tissue to the invasion of *T. pallidum* in congenital syphilis lagged behind the appearance of masses of spirochetes about

and within the tooth buds and became particularly evident after birth. Referring to the inability of the early fetal organism to defend itself, Ekehorn [15] said rightly that the spirochetes of syphilis do not need much energy for self defense; all they have to do is to nourish themselves, to multiply and to spread. However, towards the end of intrauterine life, particularly in postnatal life, the response of the tissues to the organisms became evident and reached its climax when they had degenerated. The reaction was seen in the tissues about and within the tooth buds, in the marrow and the periosteum of the jaws. All these tissues demonstrated a productive syphilitic inflammation about the vessels with a distinctive number of plasma cells and lymphocytes and a few large mononuclear cells. The tooth sacs especially were turned into a dense fibrous tissue with plasma cells and lymphocytes, occasionally showing accumulations of polymorphonuclear cells and other exudate. A very marked fibrosis with patchy masses of collagen was usually seen in them. Subsequently, degenerative changes were observed in the enamel epithelium, here and there leading to the complete destruction of the ameloblasts. As a result, formation of abortive enamel and also cessation of enamel production occurred.

The pulps were converted into fairly dense fibrous tissue with a moderate perivascular infiltration and their odontoblasts degenerated less frequently and to a far less degree than the ameloblasts. In one case the odontoblasts had disappeared and circumscribed areas of round cells with spotty necrosis, surrounded by fibrous tissue, were observed in the pulp.

None of the cases studied revealed any systemic disturbance of the calcification of the dentin and bone. The dentinoid and osteoid zones were of normal thickness. The broad layer of periosteal bone was the result of syphilitic periostitis. The new bone trabeculae, developed within the dental sac, were formed by an excessive yet irregular calcification of collagenous tissue.

It is the chronic, extremely productive syphilitic inflammation of the tooth sac that damages or destroys the ameloblasts; thus producing enamel hypoplasia. It is also this inflammatory reaction in the tooth sac that exerts pressure upon the early tooth bud and brings about the alteration of its shape.

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DESCRIPTION OF PLATES

PLATE 52

FIG. 1. Radiographs of the tibia and fibula of a stillborn syphilitic infant showing periostitis and moderate osteochondritis.

FIG. 2. Spirochetes (*Treponema pallidum*) in the reduced enamel epithelium and the tooth sac of a stillborn syphilitic infant. × 1300.

FIG. 3. Spirochetes surrounding the vessels of the outer enamel epithelium. × 1300.

1

2

3

Bauer

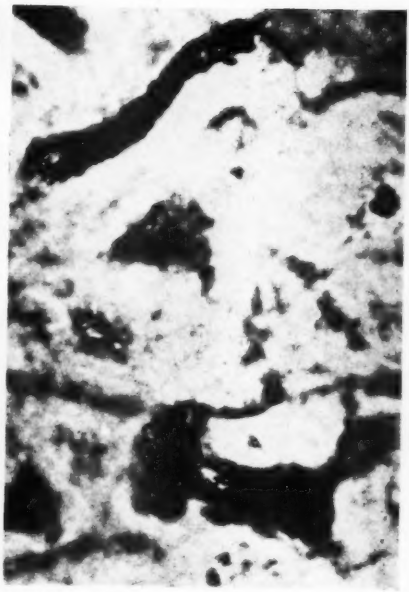
Tooth Buds and Jaws

PLATE 53

FIG. 4. Spirochetes in pulp and predentin. × 1140.

FIG. 5. Spirochetes enclosed within osteocytes of syphilitic periostitis. × 1400.

FIG. 6. Syphilitic reaction in the tooth sac of a syphilitic infant, 1½ months old. Collection of plasma cells and a few lymphocytes. × 700.



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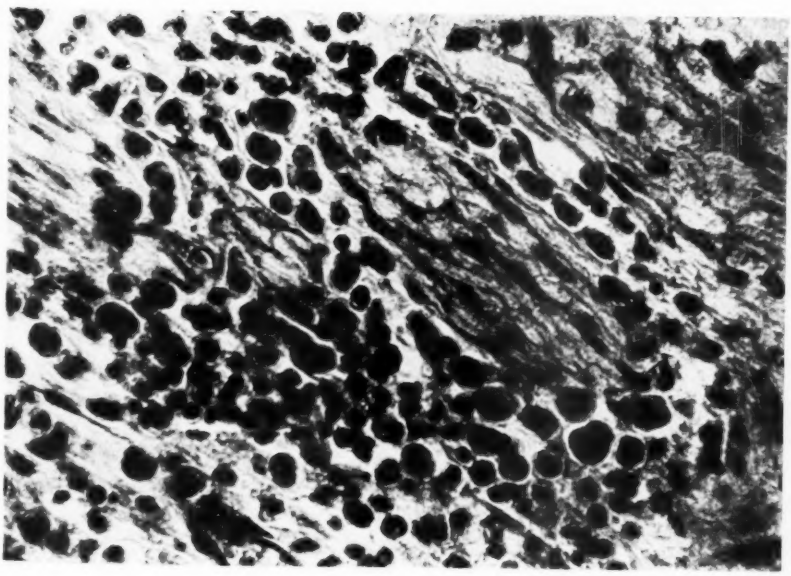

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PLATE 54

FIG. 7. Syphilitic productive inflammation in the tooth sac of an infant, 1½ months old, showing plasma cells, lymphocytes, fibrosis and excessive calcification of fibrous tissue. × 60.

FIG. 8. Dense perivascular syphilitic infiltration and collection of exudate in the tooth sac of a syphilitic infant, 1½ months old. × 60.

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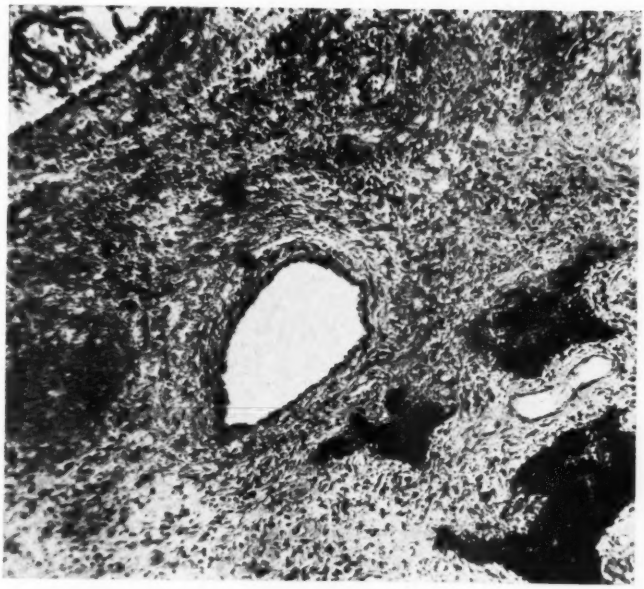

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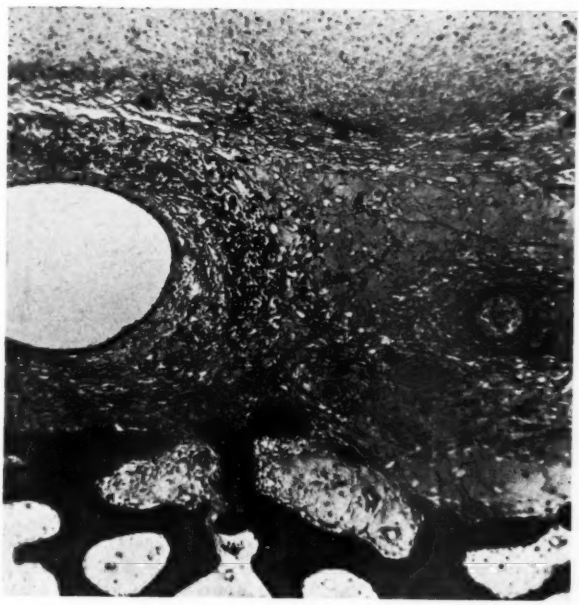

Bauer Tooth Buds and Jaws

PLATE 55

FIG. 9. Mesiodistal section through the deciduous cuspid and molar buds of a syphilitic infant, 1½ months old. E = exudate between enamel and enamel epithelium, which detached and destroyed the latter. Syphilitic inflammatory reaction throughout the bone marrow. × 4.

FIG. 10. From a syphilitic infant, 1½ months old. Abortive enamel (A) and cellular débris between enamel and degenerating ameloblasts. The predentin is of normal thickness and structure. Dentin (D) is well calcified. × 70.

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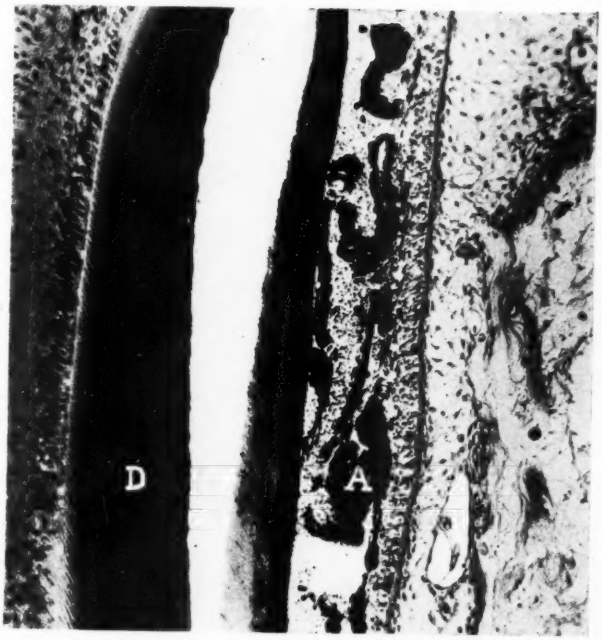

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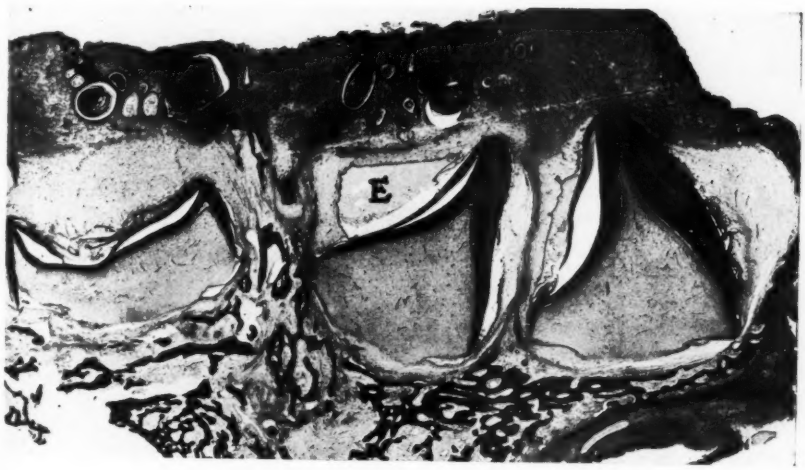


Bauer

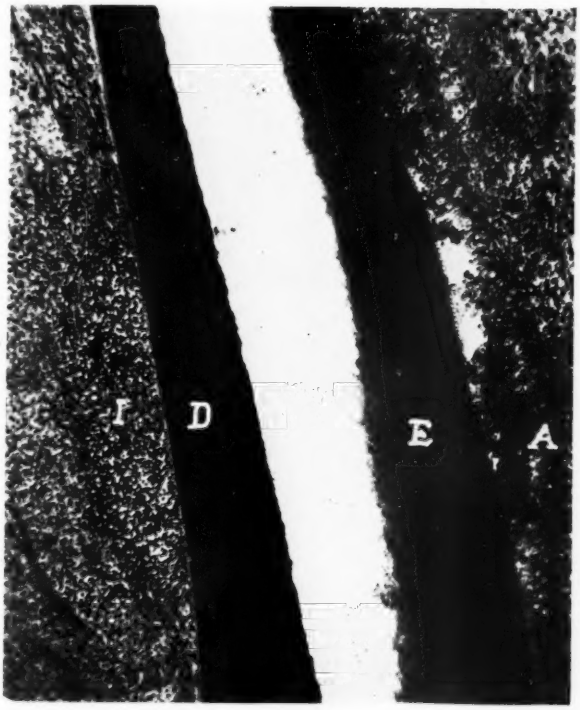
Tooth Buds and Jaws

PLATE 56

FIG. 11. From a syphilitic infant, 2½ weeks old. Accumulation of small round cells with spotty necrosis (I) in the pulp adjacent to the very thin predentin. There is no disturbance of calcification of dentin (D) and enamel (E). Abortive enamel is found between the normal enamel and detached ameloblasts (A). × 70.

FIG. 12. From a stillborn syphilitic fetus, of 9 months' gestation. Productive inflammation in the tooth sac of the lower second deciduous molar. Destruction of Hertwig's sheath. Endosteal formation of fibrous, densely calcified bone encroaching upon the tooth sac and the mandibular canal (C). The bone marrow is replaced by granulation tissue. × 40.

11



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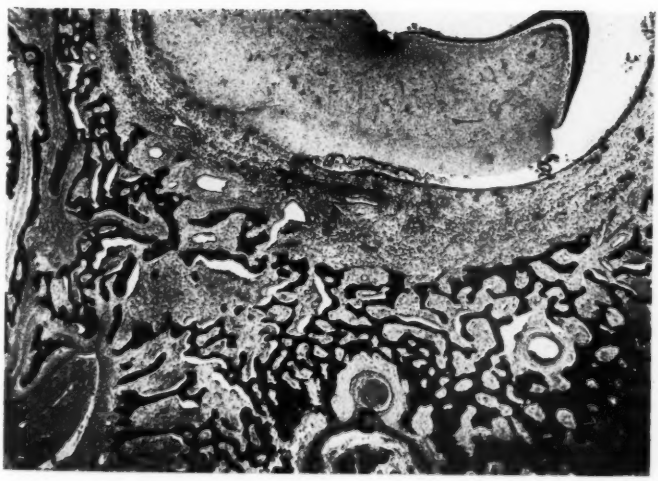

DEFECT OF ENDOCARDIAL CUSHION DEVELOPMENT AS A SOURCE OF CARDIAC ANOMALY

A Presentation of Four Cases from Autopsy Reports *

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Of the three more common cardiac anomalies, persistent foramen ovale, persistent ductus arteriosus and incomplete interventricular septum, the latter is of the most obscure embryologic origin and most often associated with other examples of anomalous cardiac development. As early as 1814, Farre [1] called attention to the frequency of occurrence of pulmonary stenosis and overriding aorta with defect of the interventricular septum. To explain all defects of the septum as a failure of the septum membranaceum to form normally is to overlook or disregard those cases where concomitant anomalies or the extent and position of the defect forces one to assume a developmental failure of much earlier origin.

It is the object of this paper to present four cases of congenital cardiac anomaly which, though differing considerably in detail and extent, seem best explained as a failure of development, in varying degree, of the ventral endocardial cushion, a structure appearing quite early in the formation of the mammalian heart.

Reports of Cases

Case 1 (Autopsy No. 1481)

The patient was a female infant, 4 months of age (though so small as to appear as newborn). She had six brothers and three sisters living and well. Four days before admission to the hospital the child began to cry frequently as though in pain. She also began to cough, and had a temperature of 104° to 105° F. The child had gained very little weight since birth and also had failed to gain in stature. There was consistent difficulty in feeding. The physical examination showed extensive consolidation in both lung fields, decreased motion of both sides of the chest, and scattered coarse, moist râles.

The heart was removed *en bloc* with the lungs and was not weighed. It was tremendously enlarged, filling (in transverse diameter) three-fourths of the chest cavity. Its largest dimensions were: length, 6 cm.; width, 5.5 cm.; anteroposterior diameter, 3.5 cm.; and circumference, 15 cm. (measured after partial fixation). The right atrium was much dilated as compared to the left; so was the right ventricle, but to a

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lesser degree. There were no serious anomalies of the great vessels other than a large, patent ductus arteriosus and a marked constriction of the aortic arch proximal to its union with the ductus. From this point distally it appeared normal. The pulmonary trunk was small but otherwise normal.

Incision of the anterior wall revealed a large and patent foramen ovale. The interventricular septum appeared intact but thin and with an unusually large, thin septum membranaceum. The atrioventricular valves were normally cusped but of unequal size, the tricuspid valve being almost one-third larger than the mitral valve. Papillary muscles and trabeculae carneae appeared normal.

Further careful examination * revealed an interesting communication between right and left sides of the heart. A channel, 1.1 cm. in length and 0.3 cm. in diameter, led from the left ventricle to the right atrium. Its origin was a slit-like opening in the superior portion of the interventricular septum, below and between the orifice of the mitral valve and that of the aortic root. The course was to the right, posteriorly and superiorly, through the substance of the cardiac septum. The atrial end of the passage was a similar slit, situated on the anteromedial wall of the right atrium just cephalad to the orifice of the tricuspid valve. In life it doubtless formed a communication by which arterial blood from the left ventricle could regurgitate to the right atrium, thus accounting for the hypertrophy of the right heart, the large patent ductus and the under-development of the preductal portion of the aortic arch.

Case 2 (Autopsy No. 738)

The patient was a white male infant, 5 months of age, who weighed 7 lbs. From a few weeks after birth the baby had been cyanotic. He was premature and weighed 3½ lbs. Weight gain was irregular but normal otherwise. Cause of death was given as epidemic cerebrospinal meningitis.

The heart was not weighed because it was removed with the lungs and thymus *en bloc*. The pericardial sac was free of fluid and was everywhere smooth and glistening. The heart was enlarged at least 50 per cent by volume and more by weight; being in systole, the ventricular walls were very thick. There was a rotation to the left which caused the right ventricle to occupy most of the precardial area, with the left ventricle displaced posteriorly. The right atrium was greatly dilated but received superior and inferior venae cavae normally. The

* Credit is due Dr. Elizabeth Conforth for the careful examination of this heart at time of autopsy which revealed the not readily apparent anomaly.

coronary sinus was enormously dilated, covering the lower portion of the left atrium, and received an extracardiac branch from above which ran in the mediastinum on the left side. Its origin was undetermined but it was, presumably, an anomalous superior vena cava (left precardial remnant).

The aorta was found to take origin in the normal location of the pulmonary artery. It was of small caliber and gave origin to an innominate artery which curved upward and to the left. The aorta, from the origin of the innominate to the point of union with the ductus arteriosus, was a very slender segment of 0.2 cm. in diameter. Here the vessel became of normal size and seemed a continuation of the ductus arteriosus. This arch then gave origin to the left subclavian artery and continued downward normally.

The pulmonary artery arose from the left ventricle, bifurcated and proceeded normally, other than for the presence of the patent ductus arteriosus already mentioned.

Internally, the only anomalies were a large opening in the interventricular septum immediately below the orifices of origin of the aorta and pulmonary arteries and a widely patent foramen ovale.

Case 3 (Autopsy No. 1092)

The body was that of a newborn white female. The weight was not recorded, and there was no history available. Anatomical diagnosis: congenital heart disease; interstitial lobular pneumonia.

The heart and lungs were removed *en bloc*. The myocardium seemed more flabby than usual but not hypertrophied. The right ventricle was slightly dilated and the right atrium larger than normal. The tricuspid valve measured 6 cm. in circumference. From the anterior left corner of the base of the right ventricle, a small orifice, 1 cm. in circumference, opened into the pulmonary artery. Opening from the same ventricle, just superior and posterior to the pulmonic valve, was a large orifice, 4 cm. in circumference, leading to the aorta.

A large foramen ovale connected the atria. The left atrium was small. The left ventricle had a small cavity and a wall 0.4 cm. thick. The mitral valve was 5 cm. in circumference. A large septal defect, 2.5 by 1.5 cm., was found in the anterior portion of the interventricular septum just inferior to the region between the openings of the great vessels and the atrioventricular orifices. This was the only opening from the left ventricle, other than the mitral orifice. An adequate septum was found between the aortic and pulmonary trunks. A patent ductus arteriosus was found.

Case 4 (Autopsy No. 250)

The patient was a colored male infant, 8 days of age, who weighed 6 lbs. and 13 oz. The child was apparently normal at birth by cesarean operation, because of a contracted pelvis. The weight was 6 lbs., 8.5 oz. The mother was a primipara and was in labor for 56 hours. On the fifth day of life the child cried and seemed ill. On the following day, he had increasing signs of cyanosis. He vomited just before death 2 days later.

The heart was markedly dilated and somewhat globular. It weighed 25.6 gm.; was 11.0 cm. in its largest circumference; 6.6 cm. in its longest vertical diameter; 5.5 cm. in its longest transverse diameter; and 1.7 cm. in anteroposterior diameter.

A triangular incision in the anterior surface revealed a single atrium and a single, almost undivided, ventricle. The inside transverse dimension of the atrium was 5.1 cm. The superior and inferior venae cavae entered posteriorly. Slightly above the opening of the superior vena cava and 0.6 cm. to the right on the anterior surface was the opening of a single pulmonary vein. To the right of this opening there was a ridge with a few shreds of tissue attached which represented all that had developed of an interatrial septum. A bicuspid valve, 4.6 cm. in circumference, separated the atrium from the ventricle and was the only communication between them. The anterior papillary muscles were small or lacking but the three posterior muscles present were very large; the largest measured 1.5 by 0.5 cm. in its greatest dimensions. All sent their chordae to the same cusp, the large posterior one. There was no division of the common ventricular cavity, but a small ridge on the posterior wall was suggestive of a septum which failed, otherwise, to develop. Trabeculae carneae were prominent.

In the left superior wall of the ventricle was the aortic orifice with three semilunar leaflets. Its circumference was 2.8 cm. A coronary artery was given off posterior to the aortic sinus and had branches to right and left sides, 0.6 cm. from its origin. The pulmonary arteries likewise arose as branches from this common aortic trunk just distal to its emergence from the ventricular wall. A communication, 1.1 cm. in length, connected the point of origin of the right pulmonary artery with the innominate artery, which it reached 0.3 cm. beyond the origin of the latter from the aortic arch. This, obviously, was the unobliterated sixth aortic arch on the right. A second communication between the aorta and left pulmonary artery occurred just distally and was interpreted as the ductus arteriosus. It, too, was patent.

Discussion

In the development of the human heart, the endocardial cushions are among the earliest internal structures to appear. During the fourth

week of fetal life the primitive tubular heart undergoes a simple S-shaped bend due to its relatively faster growth than the space between its points of anchorage by the aortic arches, cranially, and by the septum transversum, caudally. In this stage, three distinct regions are apparent: caudally, the sinus venosus which receives all venous return to the heart; next cranially, the slightly expanded atrium; and most cranially, the major portion, the bulbo-ventricular loop. It is at this time that the future atrioventricular sulcus becomes indicated externally. Internally, in the region of this sulcus, two masses of mesoderm, arising from the mass of the future epimyocardium, appear in the midsagittal plane to lift up the endothelial lining and be covered by it. One arises dorsally, and at about the same time one appears from the ventral wall. These are the endocardial cushions. They grow toward each other to meet and fuse, forming a pillar of tissue which divides the primitive atrioventricular communication into the two future atrioventricular orifices, right and left. In addition, they serve as the caudal line of attachment for the interatrial septum, next to develop, and, during the close of the fifth and beginning of the sixth week, they serve in the same capacity for the cranial margin of the interventricular septum, then being formed.

Very promptly upon the appearance of the endocardial cushions, a pair of ridges appear internally in the cranial or bulbar portion of the bulbo-ventricular loop. These ridges run parallel to the lumen of the bulb and are slightly spiral to the axis of the lumen. Their continued development and fusion form the spiral septum which later divides the bulb into the future pulmonary and aortic roots. If we accept the dynamic theory of Spitzer,* which explains the development of cardiac structures in terms of the dynamics of the fluid which perfuses them, it seems logical that the appearance of these endocardial cushions, particularly the ventral one because of its position, has much to do with the appearance of the bulbar ridges and the bulbar septum which subsequently forms from them. Also, the direction of its formation and, possibly, its ultimate fusion with the interventricular septum are similarly determined. This latter fusion, when completed, wholly separates the primitive heart into right and left halves, except for the normal embryologic continuity through the foramen ovale in the interatrial septum.

If such a significant rôle in development may be ascribed to the endocardial cushions, and particularly to the ventral one because of its proximity to the line of fusion of the septa of the bulb and ventricle,

* For an excellent summary and evaluation in English of the works of A. Spitzer, most of which were published in German from 1921 to 1933, see Harris and Farber.[3]

it becomes possible to explain the early observation of Farre[1] and likewise the four cases with which this paper is concerned.

In the first case, the channel of continuity from the left ventricle to the right atrium obviously passed through those structures arising from the substance of the ventral endocardial cushion. Here we must assume some inadequacy or interruption of development rather than failure, since septation of this heart was otherwise complete. The large area of the septum membranaceum might indicate a possible retardation of development of the interventricular septum which, however, did not hinder its ultimate completion.

In the second case, that of a heart with anterior defect in the interventricular septum and a transposition of the great vessels, the causes of the anomalies are less evident and, yet, may be inferred in their general outline. The transposition of the great vessels was the result of the spiralling of a bulbar septum in a direction the reverse of that which normally occurs. This reversal is conceivable if we assume that, during the fourth week, the dorsal endocardial cushion appeared but no ventral one grew out to meet it. This unusual condition sufficiently affected the flow of the coursing fetal blood that, though the bulbar ridges and eventually the septum were stimulated to develop, their direction was the reverse of the normal. It is a well known fact that where complementary structures develop, a failure of one is usually compensated, more or less, by overdevelopment of the other. Hence, in the absence of a ventral cushion, the pillar of tissue which divided the primitive atrioventricular communication may have been provided entirely by the dorsal cushion. It is possible, also, that the ventral cushion may have appeared, but too late to assume its normal significance. Once established, the reversed bulbar septum sought its normal union with the interventricular septum; but in the absence (or inadequacy) of what is normally supplied by the ventral cushion, such union could not be complete. Hence we find the large anterior fault in the interventricular septum, a defect occurring not at the septum membranaceum, where final closure of the septum normally occurs, but farther anteriorly and resulting from a failure of origin at a much earlier time.

The third case offers excellent evidence for the assumption that the ventral endocardial cushion plays some rôle in the union of the bulbar and interventricular septa. Each developed; the bulbar septum, completely; the interventricular septum to the region of its normal union with that of the bulb. Dorsally, the cranial extension of the interventricular septum found a normal union with the endocardial pillar, as

shown by the presence of properly positioned atrioventricular orifices, right and left. But the ventral portion of this cranial margin did not find its normal union with the bulbar septum, at that time quite complete. Instead it moved to the left and found attachment to the left wall of the aortic root, which position gave origin to both arterial trunks from the right side of the septum or the future right ventricle. That this failure to fuse normally is due to an absence of tissue supplied by a third contributor, the ventral cushion, is further substantiated by the large fault in the interventricular septum just below the point of normal septal fusion. It may be argued that this interventricular patency was necessary to give egress from the left ventricle to the great vessels since, otherwise, the left ventricle would have been a blind pouch (see description of heart). In part, this is true; yet the large area of the defect suggests that it arose as a developmental inadequacy as well as a physiologic necessity. Such inadequacy seems best explained as a retardation of development of the ventral endocardial cushion during the sixth week of fetal life.

The fourth case is one of complete failure of septation of the primitive heart. That a dorsal endocardial cushion began to form may or may not be indicated by the ridge on the dorsal wall of the common ventricle. In any event there was not sufficient cushion development partially to divide the atrioventricular canal, right and left, hence no bulbar septum formed and neither interatrial nor interventricular septa appeared beyond the initial stages of a septum primum as indicated by the shreds of tissue found on the cranial surface of the common atrium. If this septum primum formed to any extent, it found no endocardial pillar for its caudal attachment and became abortive. There is no indication of a septum secundum appearing unless these shreds of tissue indicate an early development of both septa of the atrium which were later arrested in further development.

The retention of the right sixth arch perhaps served to augment the right pulmonary supply, as did the ductus arteriosus on the left, since pulmonary arteries and aorta had origin from a common, undivided bulb.

The scant development of this heart beyond a primitive fetal stage indicates an early failure of those structures to appear which initiate the later developments leading to final septation—the endocardial cushions. Such failure must have occurred early in the fourth week of fetal life.

Summary and Conclusions

Four cases of congenital cardiac anomaly have been presented, ranging in extent from a completely septate heart, with a communication

from the left ventricle to the right atrium, to a primitive, two-chambered heart with undivided atrium and ventricle and a common pulmono-aortic bulb. These cases have been discussed and an attempt has been made to estimate the source and time of failure in fetal life.

From the facts of these cases a few general conclusions seem warranted:

1. The failure of normal endocardial cushion development leads to patency of the anterior portion of the interventricular septum which defect may be associated with anomalies of the great vessels or other structures.

2. The ventral endocardial cushion plays some rôle in the normal union of the interventricular and bulbar septa.*

3. Patencies of the interventricular septum are of two distinct origins: (a) a failure of the septum to close in the region of the future septum membranaceum, and (b) a failure of fusion of the bulbar and interventricular septa due to failure of contribution by the ventral endocardial cushion.

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* Since the completion of this paper, the published observations of Kramer[3] support our assumption of the rôle of the endocardial cushion in septation of the bulb and ventricle.

EFFECTS OF POTASSIUM IODIDE ON THE SKELETAL TISSUES OF GROWING MICE *

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In former investigations, we observed [1] that administration of potassium iodide for 20 days stimulated both proliferation and regression of the epiphyseal and articular cartilages in immature guinea-pigs; it also increased the resorption of the primary bony trabeculae during the growth period. These changes resembled in some respects the early changes found in the cartilage of growing mice and guinea-pigs subsequent to the administration of anterior hypophyseal and thyroid hormones.[2]

The present investigation was undertaken in order to determine how prolonged administration of potassium iodide influences skeletal development and ageing in mice, and how these effects compare with those obtained by the administration of anterior hypophyseal and thyroid hormones.

MATERIAL AND METHODS

Twenty-eight male mice were used in these experiments. Eight mice of the closely inbred strain C57, 4 weeks old, received intraperitoneal injections of 0.1 cc. of a 2.5 per cent solution of potassium iodide in distilled water. Two of these mice were injected for 4 consecutive days and sacrified the next day; the remaining 6 mice were injected for 5 consecutive days; no injections were given on the sixth and seventh day. The animals were killed in pairs after 1, 2 and 4 weeks following the first injection. Twenty mice of the closely inbred strain C_3H, 4 to 6 weeks old, were treated in the same way for periods of 4 days, 1, 2 and 4 weeks, and 2, 3, 4, 5 and 11 months. Four untreated male mice of the strain C57 and 10 of the strain C_3H (when possible, littermates) served as controls.

At necropsy, tibia and femur were removed as a whole. The growth zone at the upper tibia was selected for histological study.

* These experiments were conducted in the Laboratory of Research Pathology, Washington University, School of Medicine, St. Louis, Mo.

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Observations

The animals stood the injections well. During the first 2 weeks, the mice injected with potassium iodide gained somewhat less weight than the untreated animals. In strain C57, this deviation from the normal was more accentuated than in strain C_3H. However, after 4 weeks of injection of potassium iodide, there was no difference in the weights of the treated and the control mice.

Histological Examination

I. In Strain C57

(*a*) *Epiphyseal Disk.* After 4 days of injection of potassium iodide, the pattern of the growth zone of 4½-weeks-old mice was regular. In a single row, 4 hypertrophic cartilage cells were counted, as is normal for this age; the columnar cartilage cells were slightly increased in number; there were 10 to 12 instead of the usual number of 10. The nonoriented cartilage cells were rounded off and had undergone noticeable proliferation. The columnar cartilage cells likewise showed increased mitotic proliferation, and their conversion into hypertrophic cartilage cells was intensified.

One week after the beginning of the injections of potassium iodide, the epiphyseal disk was narrower than after 4 days of treatment, and also somewhat narrower than in untreated mice of corresponding age. The mitotic proliferation of the cartilage cells was less accentuated than after 4 days of treatment. In a single cartilage row, the number of columnar cells had fallen to 8 to 10, and that of hypertrophic cells to 2 to 3. Simultaneously, the columnar and hypertrophic cartilage cells had decreased in size, while the cartilage ground substance had increased in amount and the calcification of the cartilage was intensified. Moreover, the cartilage cells had undergone karyolysis and karyorrhexis, conditions not seen in control mice of this age. The conversion of columnar into hypertrophic cartilage cells and the replacement of the latter cells by bone were markedly accentuated.

After 2 and 4 weeks of treatment with potassium iodide, the zone of endochondral ossification showed a distinct narrowing and a heavier calcification than ordinarily. The number of columnar cartilage cells in a single row was 6 to 8 instead of 10, that of the hypertrophic cells was 2 instead of 4. Moreover, regressive changes in the cartilage became conspicuous. Several groups of adjoining cartilage cell-rows were thus affected, and thick plugs of amorphous cartilage appeared in the growth zone of 6-weeks-old mice (Figs. 1 and 2). In healthy untreated animals of this strain, a similar condition is not seen before the end of

the fourth month of life. Resorptive processes had likewise increased and had begun, in some places, to dissolve the amorphous plugs. Perforations of the epiphyseal disk, however, were not observed.

(*b*) *Subepiphyseal Layer.* After 4 days of injection of potassium iodide, the subepiphyseal zone, in which the replacement of cartilage by bone takes place, was congested. Mitotically proliferating spindle cells and epithelioid cells acting as osteoblasts filled the opened cartilage capsules and the peritrabecular tissue. After 1 and 2 weeks of injection, a partly fibrous, partly osteogenic tissue had developed. Numerous trabeculae containing nonossified or incompletely ossified cartilage were linked with each other by transverse bony bridges, but at the same time resorption of bone became accentuated. The activity of increased numbers of osteoclasts and of enlarged capillaries had caused a shortening of the bony spicules that were thickened in their proximal parts.

After 4 weeks of administration of potassium iodide, a thick transverse bony plate delimited the layer of hypertrophic cartilage cells from the bone marrow in 8-weeks-old mice. This condition is ordinarily not found before the end of the fourth month of life in animals of this strain. On account of the increased resorption of osseous tissue, the excessive amount of trabecular bone found at the earlier experimental stages had disappeared.

(*c*) *Joints.* During the first 2 weeks of injection of potassium iodide, the articular cartilage proliferated markedly by mitoses and underwent increased hypertrophy (Fig. 3).

After 4 weeks of treatment, the hyperplastic processes had decreased, whereas hypertrophy and ossification of the cartilage were still prominent. These changes were followed by, or associated with, an intensive resorption of cartilage and bone. Karyorrhexis and karyolysis of the articular cartilage cells were pronounced.

(*d*) *The Bony Shaft.* During the early stages the periosteum was vascular and loose. The spindle cells at the inner and outer surfaces of the compacta proliferated very much, and they were, in greater numbers than ordinarily, converted into osteoblastic epithelioid cells. Thus, both endochondral and periosteal ossification were increased, the maximum being reached after 1 and 2 weeks of injection of potassium iodide.

Following this period, increased cellular and vascular resorption caused a solution of the excessive amount of bony tissue seen at the earlier experimental stages. Thus, the histological structure did not differ from that seen in untreated mice of corresponding age. There were no changes in the bone marrow proper.

II. In Strain C_3H

The skeletal tissues of mice of strain C_3H exhibit ordinarily a faster rate of development and ageing than those of strain C_{57}.[3]

(*a*) *Epiphyseal Disk.* After injections of potassium iodide for 4 days, the epiphyseal growth zone of mice of strain C_3H, 4½ weeeks old, showed a greater narrowing and a heavier calcification, but less stimulation of proliferative processes in the cartilage than the corresponding animals of strain C_{57}. In a single cartilage cell-row of strain C_3H, 2 or 3 hypertrophic instead of 4, and 6 or 7 columnar cartilage cells instead of 10, were counted. As in strain C_{57}, the conversion of columnar into hypertrophic cartilage cells was intensified, and it began farther proximally than usual. The hypertrophic cartilage cells underwent an accentuated replacement by bone.

After 1 and 2 weeks of treatment, the cartilage cell-rows had shortened still more than after four injections of potassium iodide. The number of hypertrophic cartilage cells in a single row had now fallen to 1 or 2, whereas the number of columnar cartilage cells was unchanged at 6 or 7. The cartilage cells were shrunken and densely calcified (Figs. 4 and 5). The regressive changes had affected several cartilage cell-rows and amorphous plugs of cartilage appeared in the growth zone of mice 5 and 6 weeks old. Breakdown and osseous replacement of the hypertrophic cartilage had progressed so rapidly that after 2 weeks of injections typical hypertrophic cartilage cells were lacking, while the number of columnar cells had decreased to 5. The degenerative plugs had increased in number and extent.

After 1 month's treatment, larger areas of the epiphyseal cartilage had undergone regression and such a degree of calcification that a cell count could not be made. On the other hand, advancing bone marrow began to resorb the amorphous plugs (Fig. 6). Thus, in 2½-months-old mice of strain C_3H, the structural age of the epiphyseal growth zone was comparable to that of untreated mice of this strain 4 to 6 months of age.

After 2 months of injections of potassium iodide, the conditions were the same as after 1 month.

With prolonged duration of the experiment, the resorption of bone made some further progress, whereas the histological appearance of the cartilage cells had not changed as compared with the preceding stage. Four and 5 months subsequent to the beginning of the treatment, wider perforations of the epiphyseal plate were noted. After 11 months of injection, the structural age of the epiphyseal disk in mice of strain C_3H did not differ from that of control mice of corresponding age.

(*b*) *Subepiphyseal Layer.* The subchondral lamella separating the epiphyseal cartilage from the bone marrow was laid down as early as 1 week after administration of potassium iodide had begun, whereas in strain C57 a corresponding state was reached only after 4 weeks of treatment.

After 2 and 4 weeks of injection of potassium iodide, the greater part of the spicules had been dissolved, while the transverse bony plate had become more solid.

After 1 and 2 months of treatment, the thick osseous plate had become corroded on its distal side by bone marrow.

After 3 or more months, the subchondral bony lamella had thinned out, and such bony spicules as were still present were likewise in an advanced stage of resorption. After 11 months of treatment, the conditions did not deviate from those seen in noninjected animals of corresponding age.

(*c*) *Joints.* The hyperplasia of the articular cartilage was less accentuated, whereas hypertrophy and regressive changes were more pronounced than in strain C57. At later stages, resorption of bone was intensified. Hyalinized homogeneous areas were found in the articular cartilage. They apparently had replaced areas of cartilage that had undergone regression.

(*d*) *The Bony Shaft.* During the early experimental stages, the compacta was thicker than in strain C57, probably due to the greater amount of bone usually present in strain C_3H.

After 2 and 4 weeks of treatment, resorption of bone was more pronounced than was seen in strain C57. After 2 and more months of injection of potassium iodide, the resorptive processes were still more accentuated. After 5 months of treatment and later, the equilibrium between formation and resorption of bone was restored. The cortex showed the usual density and thickness.

Comment

In growing mice, the early effects of potassium iodide on the skeleton consist of a stimulation of the growth of the epiphyseal and articular cartilages. Subsequently, regression, calcification, ossification and resorption of cartilage and bone are increased, and the onset of epiphyseo-diaphyseal union is accelerated.

Potassium iodide thus promotes skeletal development as well as ageing (1) by an intensification of the growth processes, which is however, associated with a shortening of the growth period; (2) by an acceleration of the onset and progress of the second phase, in which

regressive changes predominate; and (3) by hastening the beginning of the third phase, the one during which resorption of cartilage and bone are prominent. However, with prolonged administration the ageing effect of potassium iodide decreases, and at later stages the structural age of the skeletal tissues is not different from that seen in normal old mice. Complete epiphyseo-diaphyseal union was not accomplished.

The present investigation on the effect of prolonged administration of potassium iodide thus supplements the observations made previously in immature guinea-pigs injected for 20 days. In these guinea-pigs, the stimulation of proliferation and regression of the cartilage had reached a maximum after 14 days, after which period it returned to normal.

The scanty reports on the effect of iodine on body growth are not in agreement. Cameron and Carmichael [4] did not observe any influence of sodium iodide on body weight and body length of rabbits and rats. Lipschütz and Morales [5] reported retardation of growth subsequent to the administration of potassium iodide to rats, but Hooker and Newman[6] noted no such retardation in mice. On the other hand, acceleration and increase of body growth were found in rats,[7, 8] chickens,[9] pigs [10] and sheep.[11] In growing guinea-pigs, we observed markedly increased mitotic proliferation of the epiphyseal cartilage after a change to an iodine-enriched diet. According to Hunziker,[12] children given iodine were taller than those kept on a normal diet.

The effects of potassium iodide in our guinea-pigs were much more transitory than in our mice. In the guinea-pigs, the treatment was short in relation to the duration of the growth period; it thus might have been too short to cause a more profound alteration of the curve of skeletal growth and ageing. In our mice, on the other hand, the treatment was extended through a large part of the growth period, and in some animals even far into the second and third phases of skeletal development. Furthermore, strain differences may exist in guinea-pigs similar to those observed in mice. These differences play a rôle in determining the responsiveness of tissues to potassium iodide, and the guinea-pigs used in our previous experiments may have belonged to a less responsive strain.

Mice of the slowly ageing strain C57 showed marked skeletal growth changes after treatment with potassium iodide. Conversely, mice of the more rapidly ageing strain C_3H, whose natural growth capacity was almost exhausted at the time of the beginning of the injections, showed relatively little or no increase of proliferation of cartilage. However,

regression of cartilage and resorption of cartilage and bone, that had been in progress at the beginning of the treatment, could be intensified and accelerated also in mice of strain C_3H.

The effects of potassium iodide on the skeletal tissues in mice and guinea-pigs decreased with prolonged administration. This may be due to an adaptation to this substance as Loeb [13] and Gray and Loeb [14] have observed to occur in the thyroid of rodents. The data of Mendel and Vickery [15] likewise suggest a gradual decrease in the effectiveness of potassium iodide, although the authors do not comment on this point. Their rats fed with additional potassium iodide showed a greater increase of weight and growth during the earlier stages of the experiments than untreated rats. However, the figures obtained at the end of the experiments were similar in the control and in the treated groups.

The effects of potassium iodide on cartilage and bone are comparable to those caused by the administration of anterior hypophyseal and of thyroid hormones.[2] The differences that do exist are those of degree rather than of kind. The action of potassium iodide was weaker and of shorter duration: there was less proliferation of cartilage during the first phase, the period of growth; regression of cartilage, characteristic of the second phase, was less enhanced than after treatment with either anterior hypophyseal or thyroid hormones; and while the onset of the third phase, that of predominant resorption, was accelerated, resorption did not progress so rapidly, and it was not so intensified as after treatment with the other two hormones. Moreover, potassium iodide caused less bone formation than did anterior hypophyseal hormone.

Although there exists a certain similarity between the skeletal effects of potassium iodide, anterior hypophyseal and thyroid hormones, little can be said as yet about a corresponding similarity in the mechanism underlying the action of these various substances. Potassium iodide seems to affect the cartilage for the most part directly, and not by way of the thyroid gland; in thyroidectomized guinea-pigs, it stimulates the growth of cartilage almost to the same degree as it does in animals with intact thyroids.[16] Other extrathyroidal effects of iodine have been reported by Chapman.[17] Rats show increased food utilization and water intake subsequent to the administration of potassium iodide as they do after injections of anterior hypophyseal hormone.[18] Furthermore, in young pigs, potassium iodide caused an increased retention of nitrogen,[10] a phenomenon also observed under the influence of anterior hypophyseal hormone.[19] Finally, thyroxin-like effects on metabolism and growth have been obtained with iodized proteins.[20, 21]

Summary

In growing mice, potassium iodide stimulates the progress of the three phases in the life cycle of the skeletal tissues. It increases temporarily the proliferation of the epiphyseal and articular cartilages, accelerates the onset of regression in the latter and stimulates first the formation and subsequently the resorption of bone. The skeletal effects exerted by potassium iodide thus resemble those caused by administration of anterior hypophyseal hormone and of thyroxin; but they are less marked and more transitory than the latter. As is the case also with various hormones, mice of the slowly ageing strain C_{57} are more responsive to the administration of potassium iodide than are mice of the more rapidly ageing strain C_3H.

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[*Illustrations follow*]

DESCRIPTION OF PLATE

PLATE 57

FIG. 1. Section through the growth zone at the upper tibia of an untreated mouse of strain C57, 6 weeks old. Epiphyseal disk shows regular pattern and is wide. × 120.

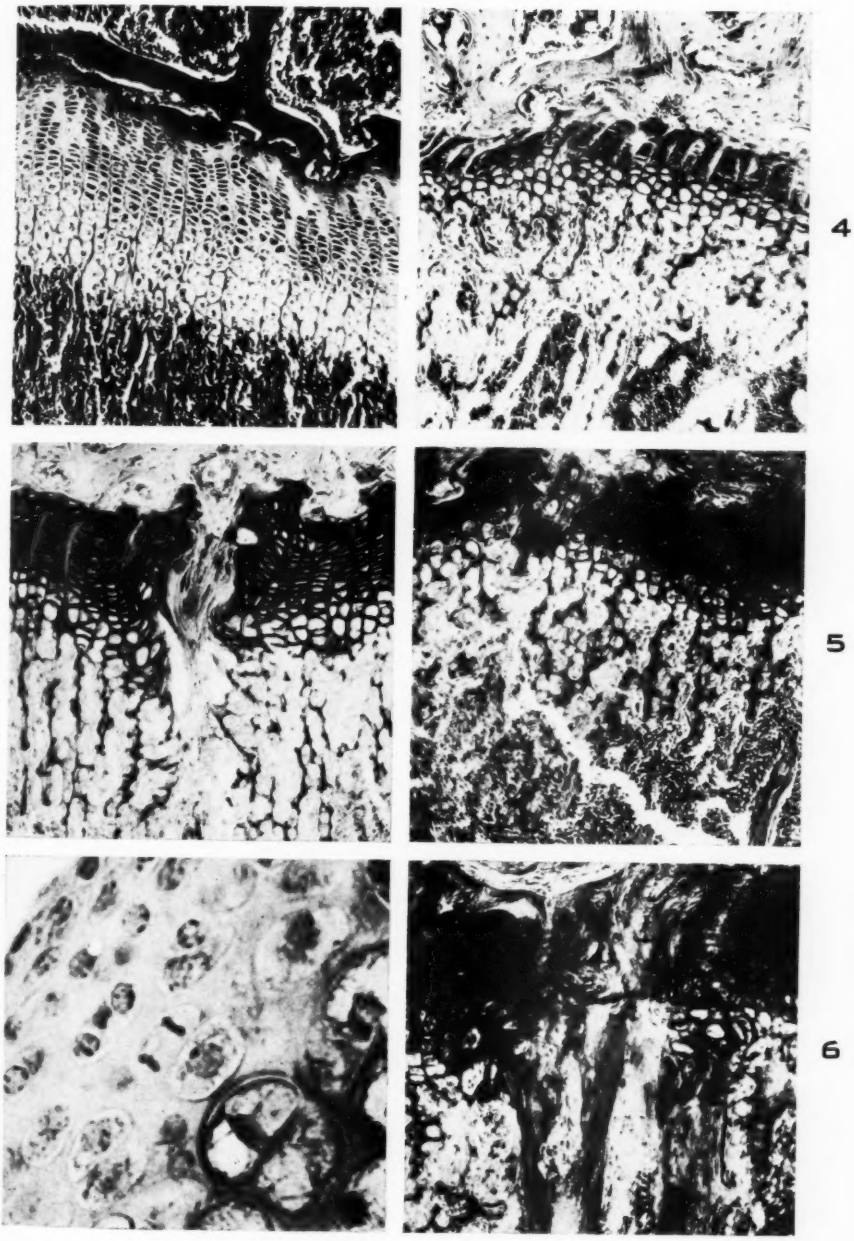
FIG. 2. Section through the growth zone at the upper tibia of a mouse of strain C57, 6 weeks old, which, from the age of 4 weeks on, had received injections of 0.1 cc. of a 2.5 per cent solution of potassium iodide five times weekly. As compared with Figure 1, the epiphyseal zone is somewhat narrowed, more heavily calcified and exhibits a thick plug of amorphous cartilage. × 120.

FIG. 3. Section through the articular cartilage of the lower femur of a mouse of strain C57, which had received four injections of 0.1 cc. of 2.5 per cent solution of potassium iodide starting at the age of 4 weeks. The articular cartilage is hyperplastic and hypertrophic and shows mitotic figures. × 500.

FIG. 4. Section through the growth zone at the upper tibia of a mouse of strain C_3H, 8 weeks old. Epiphyseal disk narrowed as compared with Fig. 1. × 120.

FIG. 5. Section through the growth zone at the upper tibia of a mouse of strain C_3H, 7 weeks old, injected for 2 weeks with 0.1 cc. of 2.5 per cent solution of potassium iodide five times weekly. As compared with Figure 4, the epiphyseal growth zone is more heavily calcified, showing formation of a plug of amorphous cartilage. × 120.

FIG. 6. Section through the growth zone at the upper tibia of a mouse of strain C_3H, 8 weeks old, injected for 4 weeks with potassium iodide. Beginning resorption of the epiphyseal plate by bone marrow. × 120.



Silberberg and Silberberg

Effects of Potassium Iodide on Skeletal Tissues

STUDIES ON AMEBOID MOTION AND SECRETION OF MOTOR END-PLATES

III. Experimental Histopathology of Motor End-Plates Produced by Quinine, Curare, Prostigmine, Acetylcholine, Strychnine, Tetraethyl Lead and Heat *

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The pharmacologist and physiologist have presented chemical and temporal evidence that chemical substances are transmitted from nerve endings to muscle. No morphologic findings are recorded in the literature, however, which support the chemical theory of transmission of the nerve impulse.

A few observations on the histologic changes occurring in the nerve endings under the influence of curare have been made. Kühne [1] described the living nerve endings in the muscle of lizards as having more distinct outlines after deep curare poisoning, and outlines still more distinct after slight curare poisoning and prolonged electrical stimulation of the nerves. Miura [2] stated that prolonged (18 days) curare poisoning in the frog caused a dwindling in the size of hypolemmal fibers. Herzen and Odier [3] found that curare caused the hypolemmal fibers of the frog to become varicose, and the axons of the nerve outside the muscle to become covered with fine granules, the change decreasing towards the center. These early observations practically exhaust the literature of studies on the effect of curare on the histologic structure of motor nerve endings.

Langley [4] presented a theory which presupposed the presence in the cell of one or more substances (receptive substances) which were able to receive and transmit stimuli, and capable of isolated paralysis, and also of a substance or substances concerned with the main function of the cell (contraction or secretion, or, in the case of nerve cells, the discharge of nerve impulses). Langley stated that his hypothesis demanded that the stimuli passing through the nerve cannot affect the contractile molecule except by means of the radical which combines with nicotine and curare. He concluded as follows:

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"And this seems in its turn to require that the nervous impulse should not pass from nerve to muscle by an electric discharge, but by the secretion of a special substance at the end of the nerve, a theory suggested in the first instance by Du Bois-Reymond."

Du Bois-Reymond [5] considered the possibility that the excitation of a striated muscle fiber through a nerve fiber might be due to the release of a chemical stimulant when the impulse arrived at the nerve ending. He stated the following:

"Von bekannten Naturprocessen, welche nun noch die Erregung vermitteln könnten, kommen, soviel ich sehe, in Frage nur zwei. Entweder müsste an der Grenze der contractilen Substanz eine reizende Secretion, in Gestalt etwa einer dünnen Schicht von Ammoniak oder Milchsäure oder einem anderen, den Muskel heftig erregenden Stoffe stattfinden. Oder die Wirkung müsste elektrisch sein."

Loewi [6] had shown that the inhibitory action of the vagus nerve on the heart was associated with a liberation of cardiac depressor substance which had a pharmacologic action on the heart similar to that of choline. He has shown also that the stimulation of the sympathetic nerves was associated with the liberation of an augmentor substance which resembled adrenalin in its action.

Dale and Feldberg,[7] through experimental evidence, have prepared the way for a consideration of an extension of the transmission of excitation by the liberation of acetylcholine, now long familiar for its peripheral autonomic effects, to all the synaptic and neuromuscular junctions of the peripheral nervous system, whether voluntary or autonomic, with the exception of the peripheral sympathetic fibers which, however, similarly employ an epinephrine-like substance, or sympathin. Brown, Dale and Feldberg [8] demonstrated that the highly potent acetylcholine was removed immediately by choline esterase. They demonstrated that there was a delay in the disappearance of acetylcholine when the choline esterase action was depressed by eserine.

The mechanism of action of quinine and prostigmine on motor end-plates in myotonia and myasthenia has become important recently in clinical medicine through the studies of the following: Wolf,[9] Kennedy and Wolf,[10] Kolb, Harvey and Whitehill,[11] Curschmann,[12] Weiss,[13] Pritchard,[14] Lindsley and Curnen,[15] Lindsley,[16] Harvey,[17, 18] Viets and Schwab,[19] Odom, Russel and McEachern,[20] and others. Harvey [17] made the following summary of his observations:

"1. The existing evidence suggests that myasthenia gravis and myotonia are due to abnormalities of neuromuscular transmission. If these are regarded as due to changes in the excitability of the motor end-plates of the muscles involved, the excitability would be lower in myasthenia and higher in myotonia than in the normal muscle."

"2. Quinine has a curare-like action by which it decreases the excitability of the

end-plates. This effect would account for its ability to improve myotonia and to increase the severity of the myasthenic state. Physostigmine and potassium chloride, as would be expected, both produce effects on these two conditions, which are in each case the opposite of those produced by quinine."

Rosenblueth, Lindsley and Morison[21] assumed:

"That the action of curare consists either in impairing the production of the mediating acetylcholine or in preventing the action of acetylcholine on the muscle, and that the decurarizing agents (physostigmine, adrenine and acetylcholine) antagonize or overcome these possible actions of curare. Thus, if curare made the muscle relatively impermeable to the acetylcholine liberated by the nerve impulses at the nerve endings, adrenine could make it permeable, injected acetylcholine could raise the concentration outside so that sufficient penetration would occur to activate the muscle, and physostigmine could achieve the same effect by preventing its destruction. These assumptions fit the data on hand. They must await, however, further evidence to test their validity."

These assumptions of specific chemical action were in accord with the evidence summarized by Cannon[22] which allowed the conclusion that the defatiguing effects of adrenine were not exclusively due to blood pressure changes, but that adrenine also had a specific effect.

In a partially curarized muscle, the excitability of the motor end-plate is lower, so that the response to nerve stimulation is reduced. When quinine is injected under these conditions, the curarization becomes complete. This action is also, in large part, responsible for the abolition by quinine of the quick response of the muscle to injected acetylcholine. Harvey[17] stated that:

"On the whole, however, my results with quinine strengthen my belief that the disturbances in these two diseases [myotonia and myasthenia] are at the motor end-plates. Such a conception gives a reasonably satisfactory explanation of the effects of various drugs on the two conditions; but there is, as yet, no clue to the fundamental processes responsible for the suggested alterations in end-plate excitability."

The studies on the after-potential and retention of negativity in nerve (Amberson and Downing,[23] Gasser and Erlanger,[24] Amberson, Parpart and Sanders[25]) have shown: (a) That reduced polarization of the nerve membrane is associated with hyperexcitability. Gasser and Erlanger found that the phase of supernormal excitability in recovery was present under the conditions which led to the appearance of after-potential, and that the two phenomena seemed to be inseparable. (b) That the passage of impulses reduces the membrane polarization, and can even depolarize it completely in crustacean nerve. (c) That such depolarization occurs readily in the absence of oxygen. (d) That oxygen appears to be necessary for maintenance of the polarization on which transmission of impulses depends, rather than for the actual transmission. Gasser and Erlanger assumed that while the spike

and refractory period are controlled largely by a chemical reaction, the after-potential and supernormality are dependent on the state of the plasma membrane of the nerve. To explain the shortening of the after-potential by cooling, a stabilization of this plasma membrane was proposed.

Through physiologic studies with the oscillograph and amplifier, Matthews[26] found that the sensory nerve endings in mammalian muscle spindles may undergo a rapid breakdown and resynthesis under certain conditions of motor nerve stimulation and temporary occlusion of the circulation. He stated the following:

"The rapid discharges which occur in the absence of an external stimulus bear a remarkable resemblance to those recorded by Adrian (1930) from damaged nerve fibres, and suggest that as a result of muscular activity and lack of circulation, the nerve ending breaks down and allows its nerve fibre to behave as though it were cut. One of the most notable features of this phenomenon is that the whole process is reversible, and that recovery from such catastrophic changes in the nerve ending can occur quite rapidly."

Matthews[26] furthermore claimed that excitable structures like nerve endings were supposed to generate a propagated disturbance by a breakdown of a polarized surface. The breakdown was followed by a refractory phase and gradual return of the membrane to its normal polarized condition. He gave evidence that when the circulation to a muscle was occluded and the nerve stimulated a number of endings "explode," due to the increased excitability and permeability of the membrane of the nerve ending. When the response of the nerve ending finally stopped due to the restriction of blood supply and high tensions of stretch, Matthews stated this phenomenon was due to a depletion of some substance necessary for repolarization inside the membrane or its accumulation outside, leading to a slowing of the recovery process. These effects were compared by Matthews with the pain occurring in man when work was done by muscles with impeded circulation (Lewis, Pickering and Rothschild[27]). Matthews stated that pain may be evoked by the rapid discharge from stretch receptors in the muscle spindles.

Morphologic evidence of "explosive" changes in the motor end-plates stimulated by either carbon dioxide or electricity has recently been presented.[28]

No histopathologic studies have been found in the literature of the effect of myotonia and myasthenia upon the motor end-plates in skeletal muscle. Such study by biopsy should be made after the normal range of variation in the size, shape and internal structure of motor end-plates in man has been established as a morphologic norm

for comparison with any abnormal changes that may be found. The morphologic effects of the chemical action of quinine and prostigmine, as well as other drugs, on the motor end-plates are relatively unknown.

The rapid changes in the pleomorphism of the secretory mechanism of the end-plate may be appreciated by others only if an adequate number of photomicrographs of the results of crucial but simple experiments are presented for study. By this method of presentation of the objective findings others may evaluate the evidence, repeat the experiments, draw their own conclusions. Furthermore, by this method, an extensive descriptive morphology is avoided.

The purpose of this paper, therefore, is the presentation of direct and conclusive experimental morphologic evidence, in the form of easily verified and clear-cut, untouched photomicrographs, that supports the following theses: (1) That the experimental pleomorphism of the hypolemmal axons of the motor end-plates is the result of normal and abnormal functional ameboid motion; and (2) That the experimental variation in the quantity of the granules of the sole plate of Kühne is the structural expression of the differential phases in the secretion of a chemical substance, possibly acetylcholine, from the terminal axons of the motor end-plates.

Materials and Methods

The motor end-plates in 10 different groups of muscle (pectoralis major, rectus abdominis, intercostals, erector spinae, biceps brachii, triceps, quadriceps femoris, biceps femoris, sartorius, and gastrocnemius) in the chameleon (*Anolis caroliniensis*), weighing 3 to 5 gm., were studied in 230 animals. The chameleon was selected for this experimental study because of the large size and clear-cut components of the motor end-plates. The normal range of variation of the motor end-plates was established for the biceps femoris muscle in 10 animals of the summer chameleon, from May to October (1941 and 1942). The muscles were subjected to various histologic technics such as the Bielschowsky method of silver impregnation as modified by Boeke[29] and by the *intra vitam* methylene blue method of Ehrlich as modified by Huber.[30] The best method for the study of the continuity of the epilemmal axon, hypolemmal axon, granular sole plate of Kühne and the cross striations of the teased muscle fibers is the modified Ranvier gold chloride technic previously described by me.[28] Over 10,000 slides of teased muscle preparations have been made for this study. Boeke claims that the periterminal network revealed in certain motor end-plates by the silver method is the morphologic counterpart of the

hypothetical "receptive substance" of Langley. The granules of Kühne, however, are best revealed in teased muscle fibers after the gold chloride method has been used. Both the hypolemmal axons and the granules of Kühne are profoundly affected by the chemical experiments employed and the histologic method adopted. In fact, the gold chloride method reveals better than any other the pleomorphism of the secretory mechanism of the motor end-plates and supports the statement that these granules could fulfil the function of Langley's receptive and transmitter substance, or they could be granules of acetylcholine or some related chemical substance. Although the hypothesis is advanced that these granules of Kühne may be acetylcholine, to date there is no reliable histochemical technic that has been devised to detect this substance. Pure acetylcholine, as well as choline, produces a granular precipitate with gold chloride in the test tube and a reliable histochemical technic for the detection of acetylcholine, based upon this fact, is now being sought.

The histologic effects on the motor end-plates, in 20 animals, of intocostrin (a purified form of curare), 1 mg. per Kg. of body weight injected intraperitoneally, are, within 10 minutes, those of retraction of 50 per cent of the hypolemmal axons and increased staining capacity with gold (Figs. 1, 28, 29, 31, and 46 to 56). The changes produced by the local injection of intocostrin (1 mg. per Kg. of body weight) into the biceps femoris muscle of 20 chameleons, followed within 3 minutes by the local injection, in the same site, of quinine hydrochloride (0.5 mg. per Kg.), and, after the lapse of 3 minutes subsequent to the injection of quinine, by another intraperitoneal injection of ammonium hydroxide (0.05 cc. of 1:100), are clearly evident (Fig. 2, and Figs. 73 to 82). The animals died in spastic rigidity within 2 minutes after the injection of ammonium hydroxide. The injection into the peritoneal cavity of prostigmine (1.0 mg. per Kg.) produced expansion in 70 per cent of the motor end-plates in the biceps femoris muscle in 20 chameleons within 5 minutes, at which time the animals were decapitated, the muscles excised and immediately prepared by the gold method (Figs. 3, 11, 12 and 30).

Quinine hydrochloride (0.5 mg. per Kg.) injected into the peritoneal cavity of 20 chameleons produced retraction in 60 per cent of the motor end-plates within 10 minutes, at which time the animals were decapitated, the muscles excised and immediately subjected to the gold method (Fig. 4). Acetylcholine (0.5 cc.; 1:1000) was injected into the peritoneal cavity of 20 chameleons and in 5 minutes the animals were decapitated and the biceps femoris muscle immediately prepared by the gold method (Fig. 5).

When tetraethyl lead (0.1 cc.) was injected into the peritoneal cavity of 20 chameleons the gold-staining material of the motor end-plates was augmented in amount and in staining capacity (Figs. 6, 7, and 83 to 87). Twenty living chameleons, with skin intact, were killed by placing them in Locke's solution at 55° C. for 10 seconds and then in Locke's solution at 4° C. for 1 minute. At 55° C. the animals became rigid within 3 to 10 seconds. Stimulation of the muscle is first through the sensory nerves from the skin and then through the motor nerves of the end-plates. Shortly following this nervous transmission of the heat stimulus, there is direct transmission of heat through the skin to the muscle (Figs. 14 and 15).

Curare (1.0 mg. per Kg.) injected intraperitoneally in 20 chameleons was followed within 5 minutes by the local injection into the biceps femoris muscle of quinine hydrochloride (0.5 mg. per Kg.). This double injection, consecutively timed, was repeated within 2 hours. One hour after the last injection the animals were decapitated, the muscles excised and immediately subjected to the gold method (Figs. 16 to 27). Strychnine sulfate dissolved in Ringer's solution free of HCO_3 and PO_4 was injected into the peritoneal cavities of 20 animals in concentrations of 1:1000 every 6 hours for 48 hours and in amounts of 0.05 cc. at each injection. The animals grossly manifested increased excitability to mechanical tapping throughout the period of 48 hours. They were then killed within 2 minutes by a lethal dose of strychnine injected into the peritoneal cavity (0.5 cc., 1:100), (Figs. 34 to 35).

Prostigmine (1.0 mg. per Kg.) was injected into the peritoneal cavity of 20 chameleons 1 minute after the onset of paralysis from curare (1.0 mg. per Kg.) which had been injected locally into the biceps femoris muscle (Figs. 57 to 59, and 60 to 63). The animals were decapitated 1 minute after the injection of prostigmine. Acetylcholine (0.5 cc., 1:1000) was injected into the peritoneal cavity of 20 chameleons 1 minute after the onset of paralysis from curare (1.0 mg. per Kg.) which had been injected locally into the biceps femoris muscle (Figs. 64 to 68, and 69 to 72). The animals were decapitated within 1 minute after the injection of acetylcholine when they were in a state of strong muscular spasm.

Results: Experimental Morphology

1. *The Pleomorphism of the Normal Motor End-Plates*

The length of the normal motor end-plates measured in the long axis of the muscle fibers in the biceps femoris muscle of the decapitated chameleon varied from 20 to 155 μ. The breadth was from 25 to 65 μ and the thickness from 10 to 30 μ. A statistical study of the frequency

in distribution gave the mean for the length of 1000 normal motor end-plates, 87.6 μ, and the mean for the width, 32.9 μ. The mean for the diameter of 1000 muscle fibers was 86.6 μ. The extremes of variation in the diameter of the muscle fibers were from 30 to 170 μ. The morphology regarding size and shape of the motor end-plates in the normal, therefore, was highly variable (Figs. 8, 9, 10 and 13). The amount of the granules in the sole plate of Kühne likewise changed. The granules were condensed in the normally retracted end-plates (Figs. 8 and 9) and they were in close relation to the hypolemmal axon. There was an increased staining capacity for gold. In the normally expanded hypolemmal axons (Figs. 10 and 13) the granules were less in amount and more dispersed. Under these conditions, the granular sole plate of Kühne had a decreased staining capacity for gold. The high coefficient of variation in the size of the motor end-plates in the different fibers of a single muscle and the bizarre shapes assumed by the hypolemmal axons have been explained previously on the basis of functional ameboidism.[28] The variations in the amount and staining capacity with gold of the granular sole plate of Kühne likewise were assumed to be due to different phases in the secretory activity of the motor end-plates inhibited by the death of the animals and the histologic technic employed.

2. The Experimental Ameboid Retraction of Motor End-Plates

Either curare or quinine produced, in many of the end-plates, an increased staining capacity for gold and an increased amount of granules in the sole plate of Kühne (Figs. 1, 4, 28, 29, 31, and 46 to 51). Quinine appeared to augment the action of curare by the increased retraction, accumulation of Kühne's granules, and staining capacity for gold, of the hypolemmal axon of the motor end-plates (Figs. 16 to 27). By the combined actions, occurring consecutively, of curare and quinine, these end-plates had a more definitely circumscribed border than normally and stood out clearly due to the retraction of the hypolemmal axons and localized condensation of the gold-staining substance. The combined action of these two chemicals appeared to form a dense, impermeable, precipitation membrane which inhibited the dispersion of the granules of Kühne into the protoplasm of the muscle fiber. Within 2 hours after the localized injection of curare followed by quinine, in repeated doses, there was a striking retraction into ball-like and oblong-shaped masses in 65 per cent of the motor end-plates. The mean for the length of 1000 of these motor end-plates was 49.5 μ (the mean for the normal length was 87.6 μ) and for the

width, 25.4 μ. The mean for the diameter of 1000 muscle fibers was 62.5 μ. Under the combined actions, therefore, of curare and quinine there is a reduction in size of a great number of the motor end-plates which is in contrast to the average increase in size of the motor end-plates in the same muscle but in different animals under the influence of prostigmine. Certain elongated motor end-plates, under the influence of the chemical action of curare during the expansive phase of ameboid motion, had a broad, dense rim of the granules of Kühne. The external border of the granular sole plate of Kühne was in direct continuity with the dark cross striations of the muscle fiber or had a festoon shape influenced by these striations. In some of the expanded end-plates, dense islands of Kühne's granules were found within the end-plate as well as condensed streamers that extended for a considerable distance into the protoplasm of the muscle fiber (Figs. 46 to 51).

3. The Experimental Ameboid Expansion of Motor End-Plates

Within 5 minutes after the intraperitoneal injection of prostigmine, there was an expansion in over 70 per cent of the motor end-plates. The mean for the length of 1000 of these motor end-plates was 110.5 μ (whereas the normal was 87.6 μ) and for the width, 54.6 μ in the biceps femoris muscle (Figs. 3, 11, 12 and 30). Fragmentation of the hypolemmal axon into discrete globules was evident in many of the end-plates expanded by the chemical action of prostigmine (Figs. 12 and 30). The morphologic effect of quinine (Fig. 4) was comparable to that of curare (Fig. 1), whereas the action of prostigmine (Fig. 3) was quite comparable to that of acetylcholine (Fig. 5) on the motor end-plates in the biceps femoris muscle. Quinine reduced the average size of the motor end-plates by ameboid retraction of the hypolemmal axons whereas acetylcholine increased the average size of the motor end-plates in the same muscle but in different animals by stimulating the expansive phase of the ameboid motion.

When the living chameleons were plunged into Locke's solution at 55° C. for 10 seconds, and the effect of this short duration of heat was suddenly stopped by plunging them into Locke's solution at 4° C., there was an expansion of 70 per cent of the motor end-plates. Most of these motor end-plates that had been expanded by heat had a diminution in the amount of immediately related Kühne's granules. These granules appeared to be dispersed into the muscle substance in relation to abnormal waves of heat rigor. Many of these heat rigor waves in the muscle substance were in direct relation to the expanded motor end-plates from which the waves appeared to radiate into the

muscle substance. The chemical action of the dispersed granules of Kühne appeared to influence the morphology of the muscle fiber by the replacement of the coarse cross striations by fine ones closely spaced. The stimulus of heat appeared to have a neoformative influence on the end-plate which underwent ameboid expansion coincident in time with the production of heat rigor in the muscle fiber. The reversible replacement of fine and coarse cross striations in muscle appeared to be the structural expression of the underlying reversible chemical changes of metabolism in normal motion. In abnormal heat rigor, these changes were irreversible.

Radiation of fine cross striations from the motor end-plates was not the result of the mere mechanical approximation of preformed coarse striations. If the motor end-plate had a constant relationship to a fixed number of preformed mechanically static membranes or units called sarcomeres, one would expect to find these striations more widely separated when the end-plate expanded and more closely approximated when the end-plate retracted. The reverse of this, however, appeared to be the case, for the coarse, widely spaced striations occurred in the small retracted end-plate, whilst fine, closely spaced striations occurred in the expanded motor end-plate. This evidence supports the statement that the coarse, widely spaced cross striations were related to the relaxed state of the muscle fiber and the fine, closely spaced ones to the contracted state of the fiber or to the condition of contracture.

4. The Experimental Material Exhaustion of Motor End-Plates

When sublethal doses of strychnine sulfate were injected into the peritoneal cavity every 6 hours, for a period of 48 hours, there was a gradual decrease in size of the motor end-plates in the biceps femoris muscle (Figs. 32, 33, and 34 to 45). This prolonged stimulation and chemical fatigue resulted in a depletion of gold-staining substance in both the epilemmal and hypolemmal axons. There was, likewise, a gradual depletion to the point of complete absence of the granular sole plate of Kühne in these exhausted motor end-plates. In fact, the exhausted motor end-plates assumed the morphology of the grape-like terminals (*terminaisons en grappe*) rather than that of the plate-like terminals (*terminaisons en plaque*). The mean for the length of 1000 of these exhausted motor end-plates was 28.4 μ (whereas the normal was 87.6 μ). The extremes for the length were from 9.5 μ to 88.4 μ. Wilkinson[31] thought that the grape-like motor end-plates were immature ones. These grape-like terminals are, however, either the result of exhaustion or quick depletion due to extreme activity and dispersion of granules.

The ramifications and reticulations of the end-plate were highly variable. In some plates there was complete globular fragmentation of the hypolemmal axons. Many of these axonic droplets were discrete and discontinuous with the main group of branches of the hypolemmal axons (Figs. 33 and 38 to 45). The grape-like exhausted motor end-plates were found in 20 per cent of the muscle fibers (Figs. 43 to 45) whereas in the muscle fibers of the normal biceps femoris muscle these depleted end-plates were found in less than 0.25 per cent of the muscle fibers. The striking contrast in size of the epilemmal and hypolemmal axons, and granular sole plate of Kühne, in the nonexhausted motor end-plates and in the exhausted ones (Figs. 43 to 45) is evident. The substantial depletion of the motor end-plates by prolonged stimulation with strychnine was comparable to that produced by the prolonged effect of anoxia caused by carbon dioxide, sodium cyanide, and fatigue due to extensive muscular exercise of the living chameleons.

5. The Experimental Formation of Acute Retention Cysts in Motor End-Plates

When curare and quinine had produced an effective block to neuromuscular transmission, the injection of ammonium hydroxide augmented the gold-staining material in both the epilemmal and hypolemmal axons because of the production of acute retention cysts. Coincident with the formation of these dilated cysts filled with the gold-staining material, there was a diminution in the size of the pseudopod-like hypolemmal axons and in the amount of related granules of Kühne. In fact, around the cysts of the hypolemmal axons there was a complete absence of the granules of Kühne (Figs. 2, 73 to 82). There appeared to be a thickening of the membrane enclosing the dilated cysts in both the hypolemmal and epilemmal axons (Figs. 74 to 77, and 81). By these chemical means, therefore, a mechanical block appeared to be formed to the secretion of the transmitter substance. This was roughly analogous to the damming back of the flowing water in a stream resulting in the formation of a lake. These axonic lakes constitute additional evidence for the thesis that the motor end-plate is a microscopic gland of internal secretion which delivers its chemical product directly into the striated muscle fiber.

6. The Experimental Massive Transmission of Material to the Motor End-Plates

There was a massive conduction of axonic nerve substance into the motor end-plate following the intraperitoneal injection of tetraethyl lead. The animal went into a state of spastic rigidity within 10 sec-

onds. There was a deformation of the hypolemmal axons and an augmentation of gold-staining material in 25 per cent of the motor end-plates (Figs. 6, 7, and 83 to 87). There was a radiation of gold-staining material and a distortion of the cross striations of the muscle substance at the terminals of the abnormal end-plates. The effect of this chemical appeared to be to augment the amount of gold-staining material in the end-plate as well as to inhibit the normal dispersal of this substance. This experimental pathology appeared to be the result of a sudden "explosion" by the rapid transfer of abnormal amounts of the transmitter substance to the end-plate.

7. *The Experimental Correlation of Ameboid Motion and Secretion of Granules of Motor End-Plates*

The production of the expansive phase of ameboid motion of the hypolemmal axons by either prostigmine or acetylcholine, applied 1 minute subsequent to the failure of neuromuscular transmission induced by curare, resulted in clear-cut morphologic changes (Figs. 52 to 72).

There was an antagonism between the stimulus of expansion produced by prostigmine and acetylcholine and that of retraction produced by intocostrin, which resulted in a clear-cut demonstration of the morphology of the expanded hypolemmal axons surrounded by an abnormal increase in the quantity of the granules of Kühne. In some examples there was a more gradual dispersion of the granules of Kühne into the cross-striated substance of the muscle fiber. There was a direct transformation, in some places, of the hypolemmal axons into granules of Kühne (Figs. 55 to 59, and 62). Agglutinated streamers of the granules of Kühne were in many places cross-striated, augmented in staining capacity for gold, and found extending from the terminals of the motor end-plate into the protoplasm of the muscle fiber. The quantity of granules and the morphology of the sole plate of Kühne, therefore, were not constant, fixed and preformed structures surrounding the hypolemmal axons. The transformation of the hypolemmal axons into granules of Kühne was made evident by the stimulus of prostigmine and acetylcholine to ameboid expansion of the hypolemmal axons and by the inhibition to the dispersal of these granules of Kühne by the chemical action of intocostrin. In many places, the hypolemmal axon underwent globular fragmentation and granulation.

This relationship of ameboid motion and granular secretion of the motor end-plates was comparable to those observations made by Korschelt[32] on the ova and secreting cells of insects, by Heidenhain[33] on

the nuclei of the cells of the salivary glands, and by Huie[34] on the profound changes in the nuclei during increased activity of the secreting cells of the insect-eating marsh plant, Drosera, when the latter is fed egg albumin. The comparative histologist is familiar, therefore, with this relationship of ameboid motion and secretion.

Korschelt[32] studied chiefly the ova and secreting cells of insects. In the egg-tubes of the ovaries of *Dytiscus marginalis,* a large water-beetle, the ova are arranged in succession like a string of pearls and separated from one another by a so-called nutrient chamber. This chamber consists of cells which produce and give off nutrient material to the ova. The behavior and the position of the nuclei of the ova toward this nutrient material is very characteristic. From the chamber the nutrient material extends into the ovum in the form of a granular mass and there disposes itself in such a manner that it comes into very close contact with the nucleus. But the most interesting fact is that which makes the activity of the nucleus toward the nutrient material apparent; namely, that the former sends pointed, pseudopodium-like processes into the granular mass where the latter touches it, and only in this direction, and thus very greatly increases the surface at the place of contact with the nutrient material. If the latter completely surrounds the nucleus, the whole surface shows pseudopodium-like processes. Korschelt described a similar phenomenon, especially in regard to the nucleus, in a whole series of arthropod and coelenterate ova. The interesting behavior of the nuclei in secreting cells toward the secreted substances forms a counterpart to these phenomena of the ingestion of substance on the part of the nucleus. Here certain relations exist toward the substances produced, which are wholly analogous to those existing in ova toward ingested substances. In the eggs of certain water-bugs, Nepa and Ranatra, there occur peculiar chitinous appendages, the so-called egg-rays, which are formed by cells especially differentiated for this purpose. These cells, of which each two unite into a single cell with two nuclei, termed by Korschelt a double cell, assume a considerable size and secrete within their body a mass of chitin. The behavior of the two nuclei in this process is very characteristic. They send out toward the middle, where the secretion is taking place, numerous, frequently branched, pseudopodium-like processes, which increase the nuclear surface upon this side very considerably, while the rest of the surface remains smooth. Such enlargements of the surface of nuclei are widespread in the secreting cells of insects and show that the exchange of substance between protoplasm and nucleus in secretion must be very active.

Baum [35] found that the nuclei of resting gland cells stained much more deeply with nuclear stains than the nuclei of gland cells that had secreted strongly. This was a histologic sign that the chromatic nuclein was destroyed in secretion. In this study on motor end-plates, the differential staining capacity of the components of the motor end-plate for gold may be, likewise, a histologic sign of variations or differential phases in the secretory activity of the gold-staining granules of Kühne of the motor end-plates.

Summary

The experimental pleomorphism of the hypolemmal axons of the motor end-plates is a result of normal and abnormal functional ameboid motion. The experimental variation in the quantity of the granules in the sole plate of Kühne is the structural expression of the differential phases in the secretion of the chemical substance, possibly acetylcholine, from the terminal axons of the motor end-plates. There is a correlation between ameboid motion and the secretion of granules which had been designated, collectively, in the past as the granular sole plate of Kühne. These aurophilic granules of Kühne may be increased in quantity by chemical reagents, such as curare and quinine, which inhibit the adequate dispersal and dissolution of the secreted granules into the protoplasm of the muscle fiber. Neuromuscular transmission is blocked by the action of curare and quinine on the motor end-plates through ameboid retraction of hypolemmal axons and the formation of a dense and circumscribed precipitation membrane composed of the granules of Kühne. The aurophilic epilemmal and hypolemmal axons undergo acute dilatations through the sudden formation of retention cysts produced by the chemical action of curare and quinine followed by that of ammonium hydroxide, which excites substantial transmission to the end-plates. The secreted granules of Kühne may be decreased in quantity to the level of complete depletion by prolonged chemical stimulation such as that produced by strychnine, sodium cyanide, carbon dioxide, and exhausting muscular exercise. Under conditions of exhaustion, in addition to the absence of the granules of Kühne, there is an abnormal decrease in the size of the epilemmal and hypolemmal axons. The terminal expansions of the hypolemmal axons of the end-plate may undergo direct transformation into the secreted granules of Kühne, without the presence of the intervening clear space, by sudden stimulation with either prostigmine or acetylcholine after the end-plate has been blocked by the local action of curare. Heat produces a sudden expansion of the end-plate and a

dispersal of the granules of Kühne that together produce perturbations in the pattern of the cross striations of the muscle fiber. These waves of contracture or rigor appear to be produced by the dispersal of some chemical substance transmitted by the motor end-plate. Tetraethyl lead produces a sudden and explosive transmission of an abnormal quantity of aggregates of aurophilic granules which results in massive radiations, distortion and increased staining capacity of the end-plates for gold. There are, likewise, abnormal distortions of the related cross striations of the muscle fiber to these abnormal end-plates produced by the chemical action of tetraethyl lead. The hypolemmal axons of the end-plate and the related granules of Kühne and cross striations of the muscle fiber are not preformed, static, and fixed in morphology. Their size, shape and internal structure are correlated with physiologic and pathologic secretory activities of neuromuscular transmission.

I wish to express deep gratitude to Mr. Leo Massopust, Director of the Department of Art and Photography, for aid with the photomicrographs; to Dr. G. Kasten Tallmadge, Assistant Professor of Anatomy, for reading the manuscript; to Messrs. John Schmitz, James Keyes, Robert Jeub, Joseph Hamel and Eugene Haushalter for technical aid in the teasing of muscle and nerve plates; and finally to Dr. H. Sidney Newcomer, Medical Department, E. R. Squibb and Sons, for the intocostrin used in these experiments.

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[*Illustrations follow*]

DESCRIPTION OF PLATES

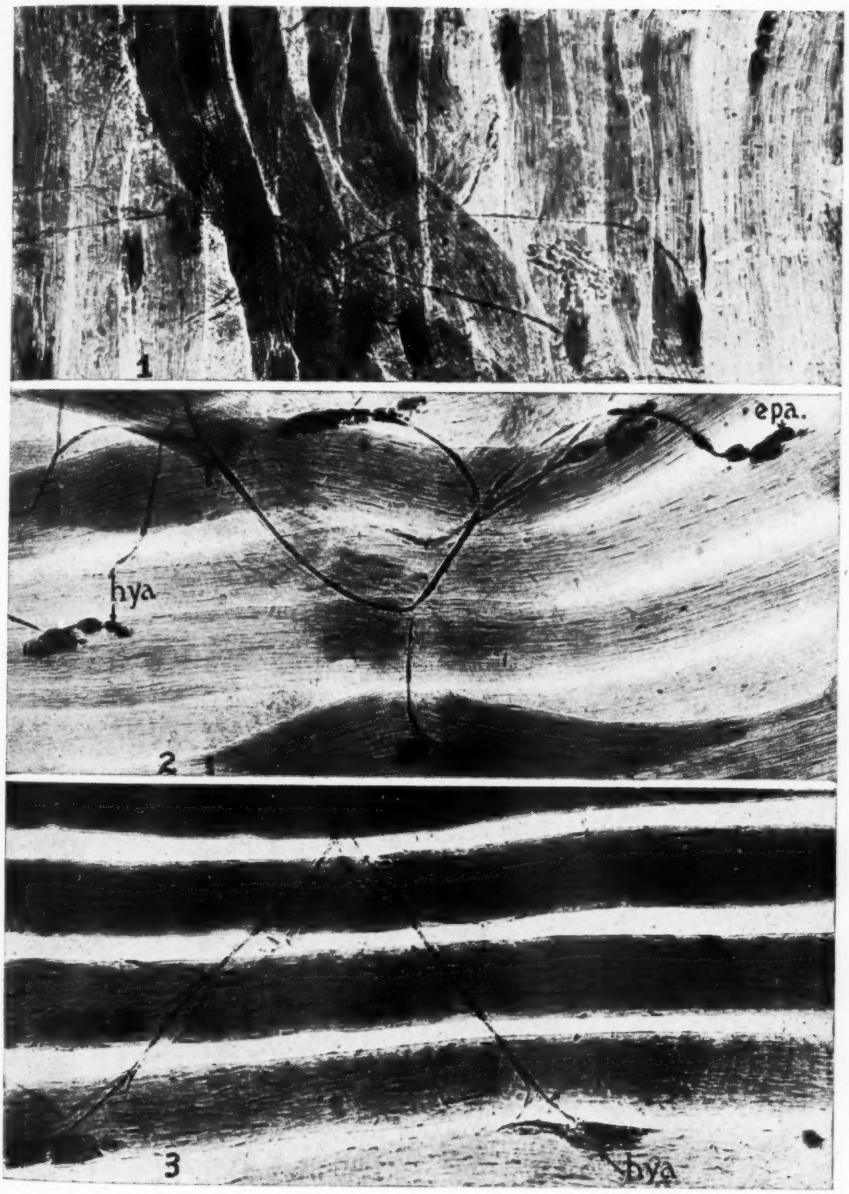
The photomicrographs of Plates 58 to 75 are from the teased whole muscle fibers (biceps femoris) and motor end-plates of the summer (May to October) chameleon (*Anolis caroliniensis*). These teased preparations of motor end-plates in skeletal muscle were previously prepared by the gold chloride technic. The photographs were prepared as direct contact prints from the negatives which were photographed under the microscope and not subjected to subsequent enlargement. In this manner, these photographs are easily comparable with those of the white rat previously published.[28] In the plates, "epa." means epilemmal axon and "hya.," hypolemmal axon. There has been no retouching of either negatives or prints.

PLATE 58

FIG. 1. Sprays of medullated nerve fibers and motor end-plates which are retracted as a result of the intraperitoneal injection of intocostrin (curare). By centripetal retraction of ameboid motion the end-plates vary in size from 24 to 85 μ measured in the long axis of the muscle fiber. The axis cylinders vary from 1 to 12 μ in diameter. × 125.

FIG. 2. Sprays of medullated nerve fibers and motor end-plates with acute cystic retention of the gold-staining axonic substance in both the epilemmal (epa.) and in parts of the hypolemmal (hya.) axons. This acute cystic retention was produced by first injecting intocostrin locally into the biceps femoris muscle and 3 minutes later injecting locally quinine hydrochloride. Three minutes after the last injection, ammonium hydroxide was injected into the peritoneal cavity. These relatively simultaneous actions of a chemical block to the secretion of the transmitter substance and the stimulating action centrally by the ammonium hydroxide resulted in the sudden accumulation of the axonic liquid substance into dilated cysts, 10 to 40 μ in diameter, of the axis cylinder both in the epilemmal and hypolemmal axons. The axis cylinder varies from 1 to 40 μ in diameter. × 125.

FIG. 3. Sprays of medullated nerve fibers and motor end-plates expanded by the action of prostigmine injected into the peritoneal cavity. By the centrifugal expansion of ameboid motion the surface area of the motor end-plates under the action of prostigmine is greatly increased. This morphologic change aids in the dissemination of the transmitter substance from its point of origin in the end-plate and its dispersion throughout the muscle fiber. The axis cylinder varies from 1 to 18 μ in diameter. The motor end-plates vary from 40 to 185 μ in length measured in the long axis of the muscle fiber. The value of the gold chloride technic in preserving the anatomic continuity of the epilemmal axon, hypolemmal axon, ramifications of the terminal axons, the granules of Kühne and the muscle striations is demonstrated not only in Plate 58 but in all of the subsequent illustrations. × 125.



Carey

Motion and Secretion of Motor End-Plates

PLATE 59

FIG. 4. Retraction of motor end-plates under the influence of quinine sulfate injected intraperitoneally. The motor end-plates vary from 35 to 85 μ in length, measured in the long axis of the muscle fiber. These end-plates have an intense affinity for gold and have in many places sharply defined, well circumscribed borders. Many of the muscle fibers are narrow in diameter, granular and coarsely cross-striated. The axis cylinders vary from 1 to 18 μ in diameter. $\times$ 125.

FIG. 5. Sprays of medullated nerve fibers and motor end-plates in the state of centrifugal ameboid expansion produced by the intraperitoneal injection of acetylcholine. The axis cylinders vary from 1 to 22 μ in diameter. The motor end-plates with multiple ameboid ramifications vary from 50 to 205 μ in length, measured in the longitudinal axis of the muscle fiber. Most of the muscle fibers are wide in diameter and have fine, closely spaced cross striations. Two of the fibers toward the left (Fig. 5) are narrow and coarsely cross striated, with a zone of transition into fine cross striations at the upper end of each fiber. These stimulated motor end-plates have a greater number of terminal dichotomous divisions than the retracted ones under the influence of either intocostrin or quinine (Figs. 1 and 4). Acetylcholine excites neurocladism or the production of new ameboid projections of the end-plate. $\times$ 125.


4

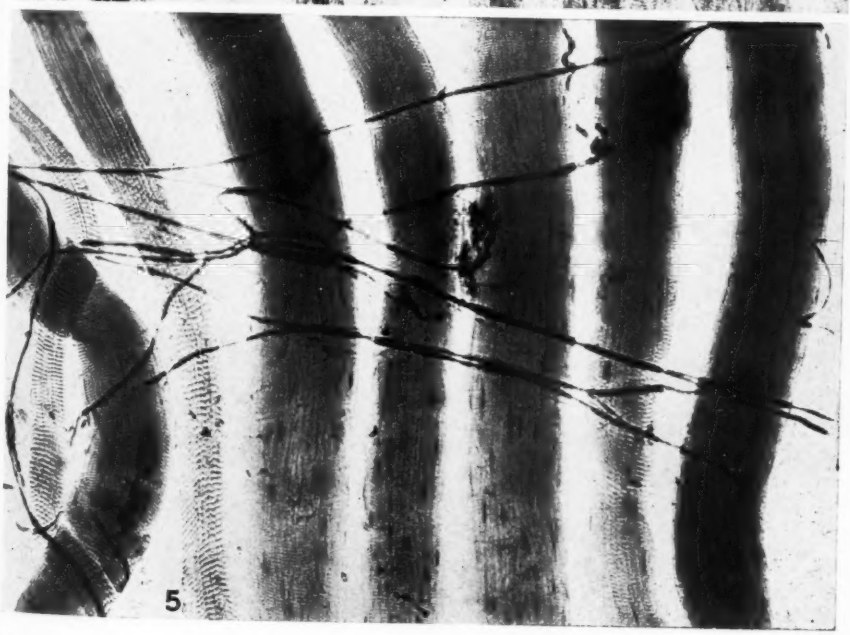
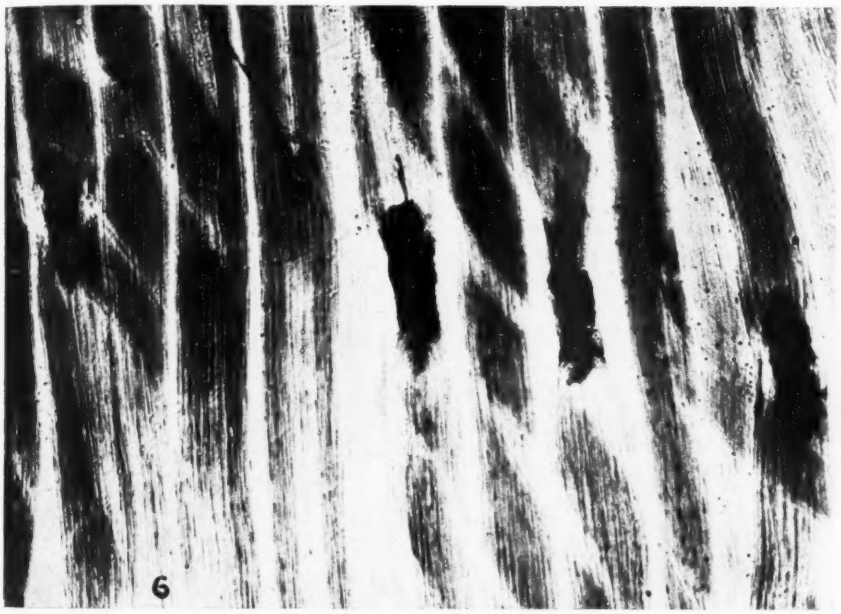

5

PLATE 60

FIGS. 6 and 7. The motor end-plates are distorted with enormous accumulations of the axonic substance which has an intense affinity for gold. This effect is produced by the intraperitoneal injection of tetraethyl lead. There are radiation rays extending from the terminals of these distorted end-plates, which plates vary in length from 80 to 285 μ. In the right half of Figure 7, one axis cylinder has three branches, two of which terminate in expanded plates and one in a distorted end-plate intensely stained with gold chloride. The epilemmal axons vary from 1 to 25 μ in diameter. $\times$ 125.



Carey

Motion and Secretion of Motor End-Plates

PLATE 61

FIGS. 8 to 10. Normal teased biceps femoris muscle fibers of the chameleon. The narrow muscle fibers with coarse cross striations (Figs. 8 and 9) have retracted motor end-plates with strong affinity for gold chloride. The wider muscle fiber in the same muscle (Fig. 10) has an expanded end-plate with multiple ramifications to which are related fine, closely spaced, cross striations. The expanded nerve plates in the active muscle fiber have variable degrees of ameboid extensions of the terminal arborizations of the axon. The hypolemmal (hya.) axons in some of the end-plates terminate in rounded or oblong swellings peripherad to which there may be a light halo-like space around which are found the granules of Kühne. The quantity of the granules in the sole plate of Kühne is highly variable, from the point of complete absence of granules to that of the accumulation of a considerable quantity of the granular material. The granules of Kühne, therefore, are a highly inconstant part of the morphology of the end-plate even in relatively normal muscle. The granules of Kühne are in direct continuity with the dark, anisotropic cross striations of the muscle fiber. The retracted motor end-plates (Figs. 8 and 9) vary from 50 to 60 μ in length and have, respectively, 18 and 16 related dark bands of the cross striations. The elongated end-plate (Fig. 10) is 150 μ in length and has 71 related dark bands of the cross striations. $\times$ 750.

FIGS. 11 and 12. Expanded motor end-plates influenced by the intraperitoneal injection of prostigmine. Another type of expanded end-plate (Fig. 30) is likewise characteristic of the effect of prostigmine, which appears to influence the centrifugal phase of expansion of ameboid motion as well as fragmentation of the hypolemmal axons into droplets of gold-staining globules. Although the granular sole of Kühne is present in many places, it appears to be undergoing rapid dispersal throughout the muscle fiber. The granules are not accumulated into densely stained islands and membranes as they are under the influence of intocostrin (curare) and quinine. $\times$ 750.

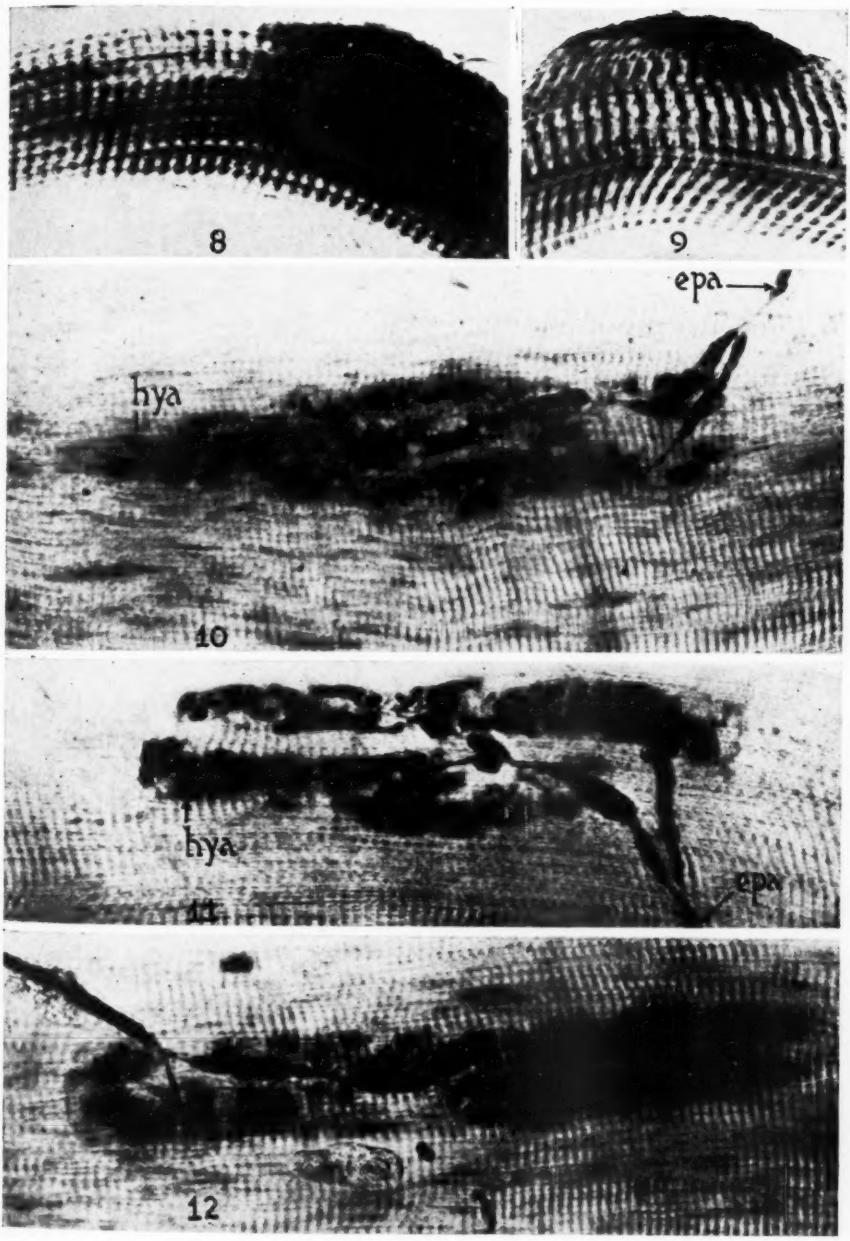
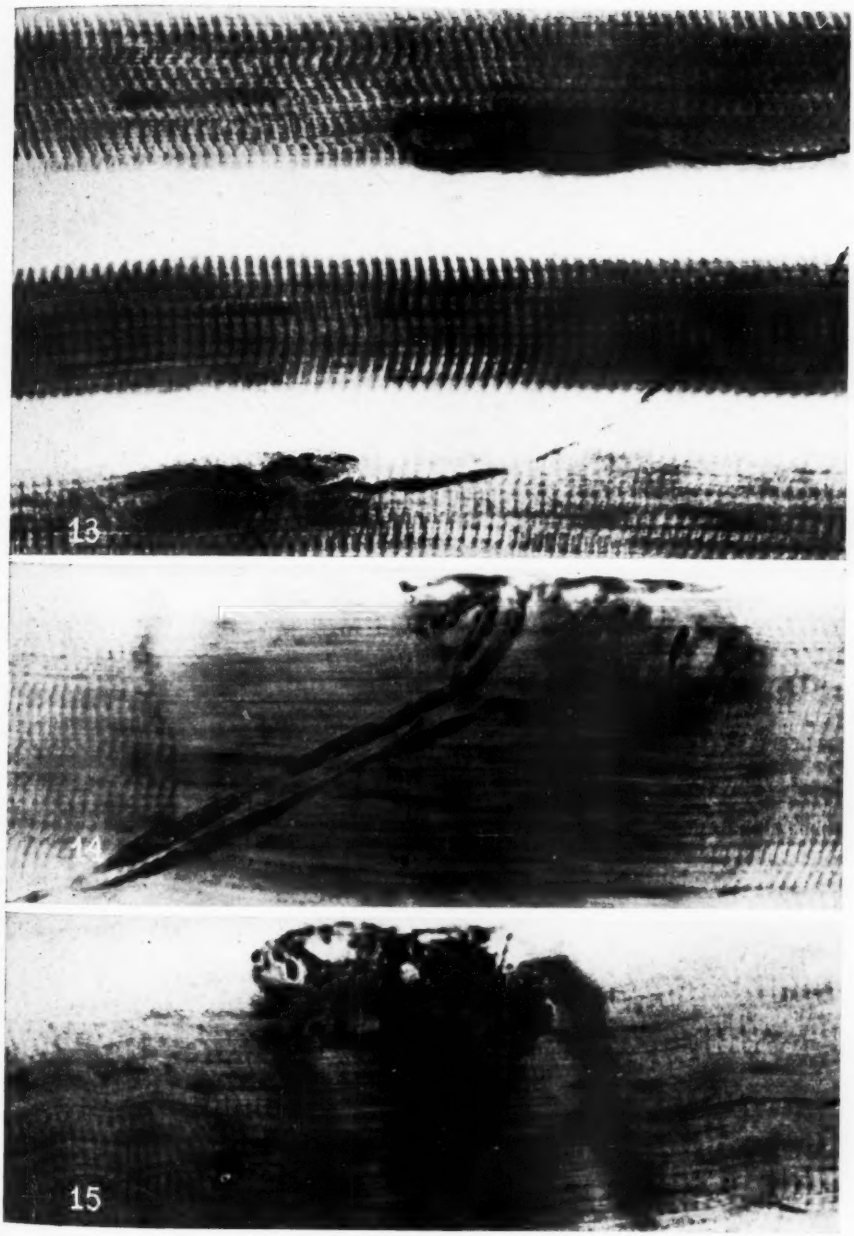

8
9
epa
hya
10
hya
epa
12

PLATE 62

FIG. 13. Two normal motor end-plates in narrow, coarsely striated fibers of the biceps femoris muscle. There are found variable degrees of splitting of the coarse striations into finer ones in these fixed muscle fibers. The axis cylinders vary from 1 to 15 μ in diameter. × 750.

FIGS. 14 and 15. Radiations of fine, closely spaced cross striations from motor end-plates quickly fixed after the living animal, with skin intact, was subjected to 55°C. for 10 seconds and was then immediately plunged in Locke's solution at 4°C. The motor end-plates, expanded by sudden changes in thermal energy, have the surrounding granules of Kühne undergoing dispersal and in direct continuity with the wave of fine cross striations radiating from the expanded end-plates. The chemical action of the dispersed granules of Kühne appears to influence the morphology of the muscle fiber by the replacement of the coarse by the fine cross striations. The transmitter substance secreted from the end-plates appears to have a profound effect on the morphology of the cross striations. This Leisegang phenomenon in a capillary space, such as that of a muscle fiber, is dependent upon temperature and on the concentration and composition of the chemical reactions. If the cross striations were preformed, constant in number and fixed in structure, and in relation to the motor end-plate, one would expect that when the so-called sarcomere is shortened during contraction the motor end-plates likewise would be shortened mechanically. The fact is that the stimulus of heat has a neoformative influence on the end-plate which undergoes ameboid expansion during the time when the muscle fiber contracts. There are a great number of fine, closely spaced striations in relation to the expanded motor end-plate. This evidence points to the fact that the fine striations of contraction and heat rigor rapidly replace the coarse ones of relaxation, and, furthermore, that the fine striations are not the mere mechanical approximation of the coarser ones. This reversible replacement of fine and coarse cross striations of contraction and relaxation respectively, giving the appearance of a shuttle-like shift, is the structural expression of the reversible chemical changes of metabolism. This occurs with flash-like rapidity and may easily mislead the observer to the conclusion that during contraction there is a mere mechanical approximation of constantly fixed and preformed membranes. × 750.

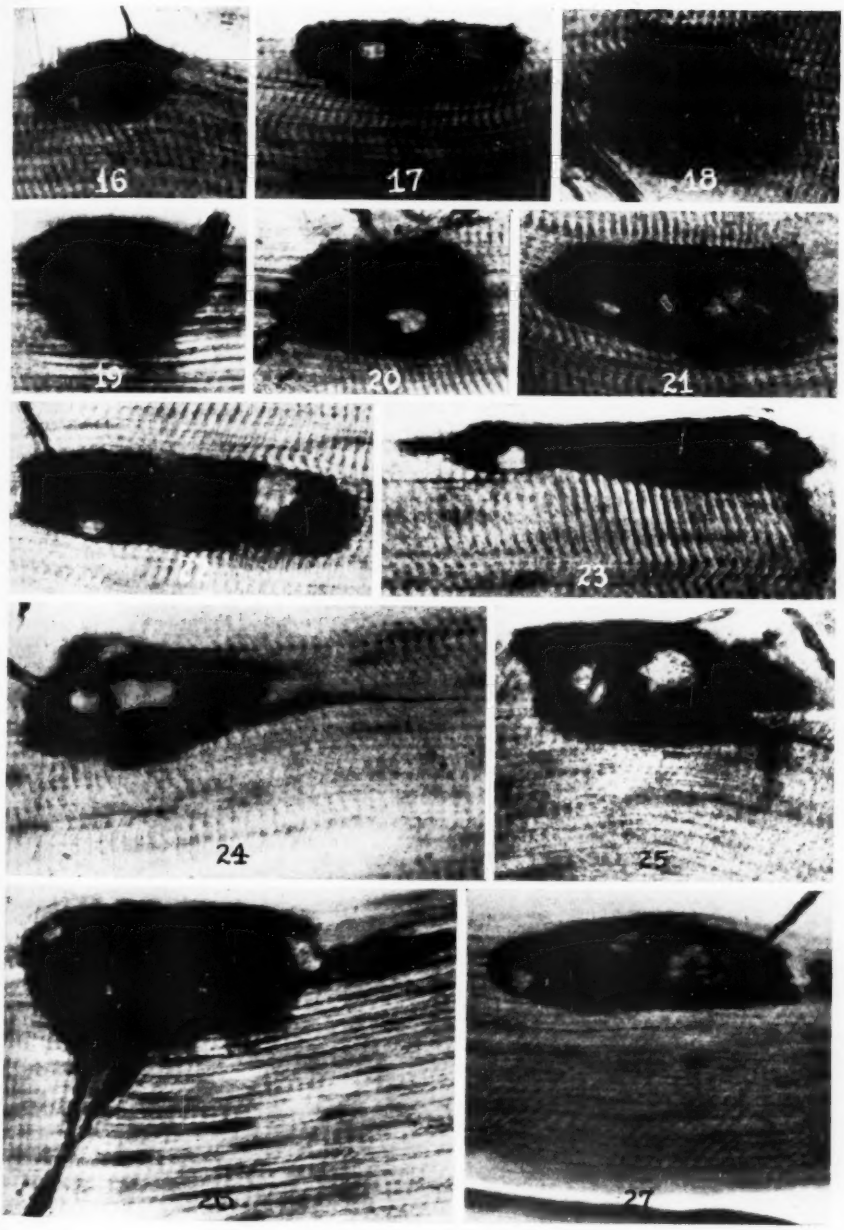


Carey

Motion and Secretion of Motor End-Plates

PLATE 63

FIGS. 16 to 27. Greatly retracted motor end-plates in the biceps femoris muscle after the injection of intocostrin locally into the muscle and of quinine sulfate into the peritoneal cavity. These retracted end-plates have a well defined circumscribed border and an intense affinity for the gold chloride. This is morphologic evidence of a localized increase in concentration of the aurophilic substance both within the terminal axons and in the region of the granular sole plate of Kühne. These granules form a condensed precipitation-membrane which gives the clearly defined border of the motor end-plate. This failure of dispersal of the secreted granules of Kühne by the chemical combination with both intocostrin and quinine, which have a strong astringent action, leads to a condensed membrane formation. The light vacuolar spaces are occupied by nuclei of the granular sole plate of Kühne. Streamers of condensed granules of Kühne are found to the right in Figures 24, 25 and 26. There appear to be conclusive findings of a morphologic nature correlated with the physiologic block in neuromuscular transmission produced by both curare and quinine. × 750.



Carey

Motion and Secretion of Motor End-Plates

PLATE 64

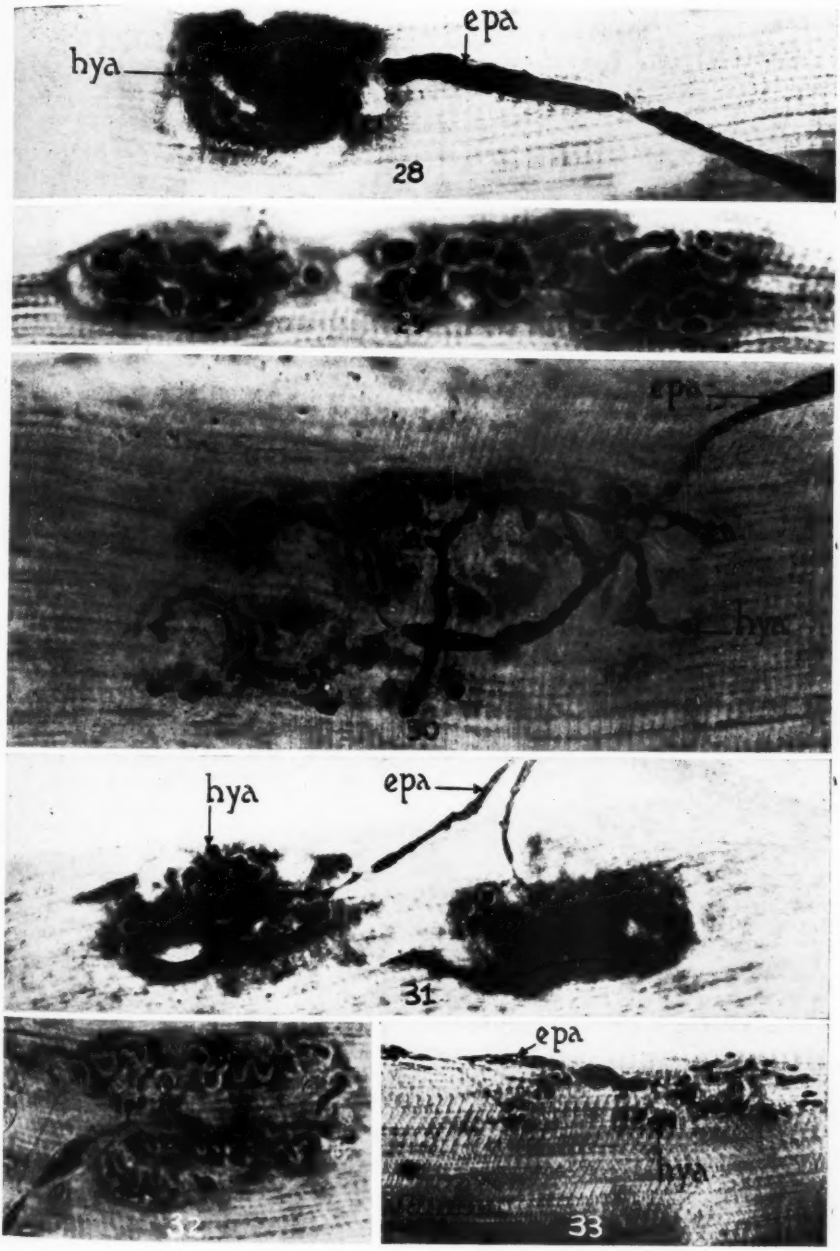
FIG. 28. Retracted motor end-plate in the biceps femoris muscle of the chameleon after the intraperitoneal injection of intocostrin. Both the epilemmal (epa.) and the hypolemmal (hya.) axons are increased in diameter from 1 to 20 μ. In the crotch of the divided hypolemmal axon there are dense islands of the granules of Kühne. × 750.

FIG. 29. Expanded motor end-plate in the biceps femoris muscle with large fragmented globules of the hypolemmal axon after the intraperitoneal injection of intocostrin. There is a clear space between the expanded terminals of the hypolemmal axon and the granular sole plate of Kühne. × 750.

FIG. 30. Expanded motor end-plate with centrifugal projection of ameboid processes and globular fragmentation of the hypolemmal axon after the intraperitoneal injection of prostigmine. In some locations there are isolated droplets of the hypolemmal axon which are completely disconnected from the main axonic network. The hypolemmal axons are thinner and have relatively less accumulation of the granules of Kühne than those under the influence of either curare or quinine. × 750.

FIG. 31. Duplex motor end-plate in a biceps femoris muscle that was first under the influence of curare injected locally into the muscle, and, 2 minutes later, excited by prostigmine injected into the peritoneal cavity. In the hypolemmal axon which appears to be secreting granules of Kühne, the axon becomes more attenuated and less in diameter than in those in which there appears to be (Figs. 28 and 29) either a failure of secretion or of dispersion of the granules of Kühne. × 750.

FIGS. 32 and 33. Motor end-plates of the biceps femoris muscle in which there is a gradual depletion of gold-staining substance in both the hypolemmal axons and granules of Kühne. These granules are practically absent (Fig. 33) after prolonged stimulation with repeated injections of strychnine sulfate over a period of 48 hours. These two end-plates were taken from neighboring muscle fibers in the same muscle. There is progressive decrease in size in both the epilemmal (epa.) and hypolemmal axons (hya.) which is morphologic evidence of exhaustion of the transmitter substance by prolonged chemical stimulation. There is complete fragmentation of the hypolemmal axons in many places into droplets (Fig. 33), around which there is either a great reduction or a complete absence of the granular sole plate of Kühne. × 750.

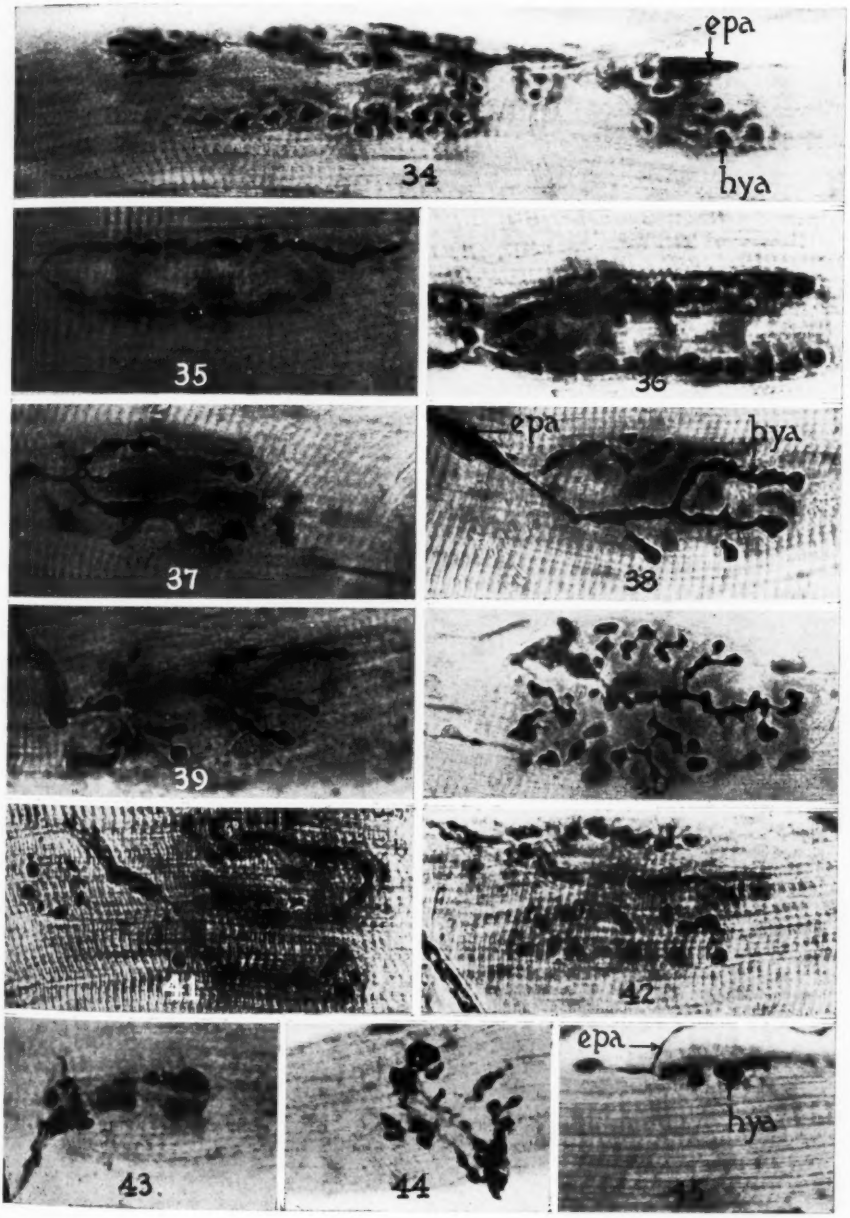


Carey

Motion and Secretion of Motor End-Plates

PLATE 65

FIGS. 34 to 45. Gradual depletion in the amount of the aurophilic substance in the epilemmal axons (epa.), hypolemmal axons (hya.) and granular sole of Kühne, in the biceps femoris muscle after repeated injections of sublethal doses of strychnine sulfate over a period of 48 hours, terminating in a lethal dose. These motor end-plates appear to be formed by ameboidism during superfunctional stimulation. The motor end-plates undergo protoplasmic streaming in a centrifugal direction into the muscle substance. The pleomorphism is as variable as that of the pseudopods of an ameba. There are dichotomous branchings and anastomotic reticulations of the terminals of the hypolemmal axons in the end-plate. In many places, the globoid and oblong terminals are completely pinched off from the main trunk of the hypolemmal axon. This progressive decrease in the amount of the gold-staining substance in the motor end-plates to the point of practically complete absence of Kühne's granules is morphologic evidence of a substantial depletion of the transmitter substance leading to exhaustion by the prolonged abnormal stimulation with strychnine. The droplet endings with an absence of Kühne's granules (Figs. 41 to 45) give a morphologic appearance of one type of motor end-plate classified by morphologists as the grape-like ending (*terminaisons en grappe*) in contrast to the plate-like ending (*terminaisons en plaque*). These endings of axonic droplets, however, were produced by exhaustion through prolonged chemical stimulation. They represent depletion of both the axonic and granular substances by abnormal stimulation. Certain endings in normal muscle that are devoid of the granules of Kühne may represent a stage in which the granules are quickly dispersed. × 750.

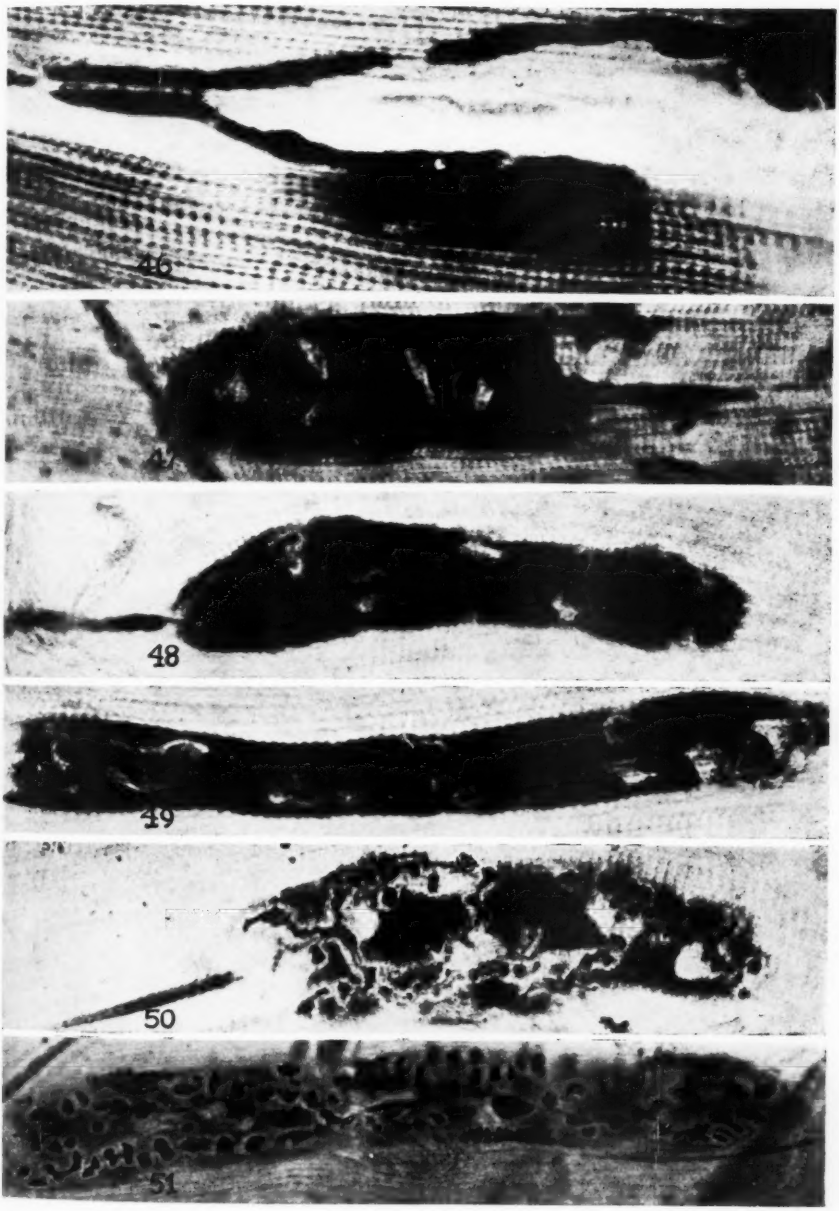


Carey

Motion and Secretion of Motor End-Plates

Plate 66

Figs. 46 to 51. Types of retracted and expanded motor end-plates produced by the intraperitoneal injection of intocostrin. The physiologic block to neuromuscular transmission is correlated with a failure of dispersion of the transmitter substance, or the granules of Kühne, from the motor end-plate to the substance of the biceps femoris muscle fiber. There is an abnormal accumulation of the condensed granules of Kühne into a thickened precipitation-membrane surrounding the hypolemmal axons of the end-plate. The accumulated granules of Kühne in the majority of the end-plates have a well defined, circumscribed border from which radiate the dark cross striations. This is evidence of failure of dispersion of the granules of Kühne into the muscle substance. In some of the end-plates (Figs. 47, 48 and 49) the definite border of the sole plate of Kühne is scalloped by the continuous relationship of the dark cross striations. Physiologic block by curare, therefore, appears to be due to an accumulation through failure of dispersal of the granules of Kühne secreted from the terminals of the hypolemmal axons. In some end-plates (Figs. 47 and 50) there are elongated and agglutinated streamers of the granules which appear to be condensed, *in situ,* by the chemical combination with curare. Curare appears to form a precipitation-membrane of Kühne's granules. This inhibits the normal transmission by diffusion and dispersion of this fulminate-like nervous substance that is secreted from the hypolemmal axons and that normally excites the muscle substance by this chemical transmission of nerve impulses. × 750.

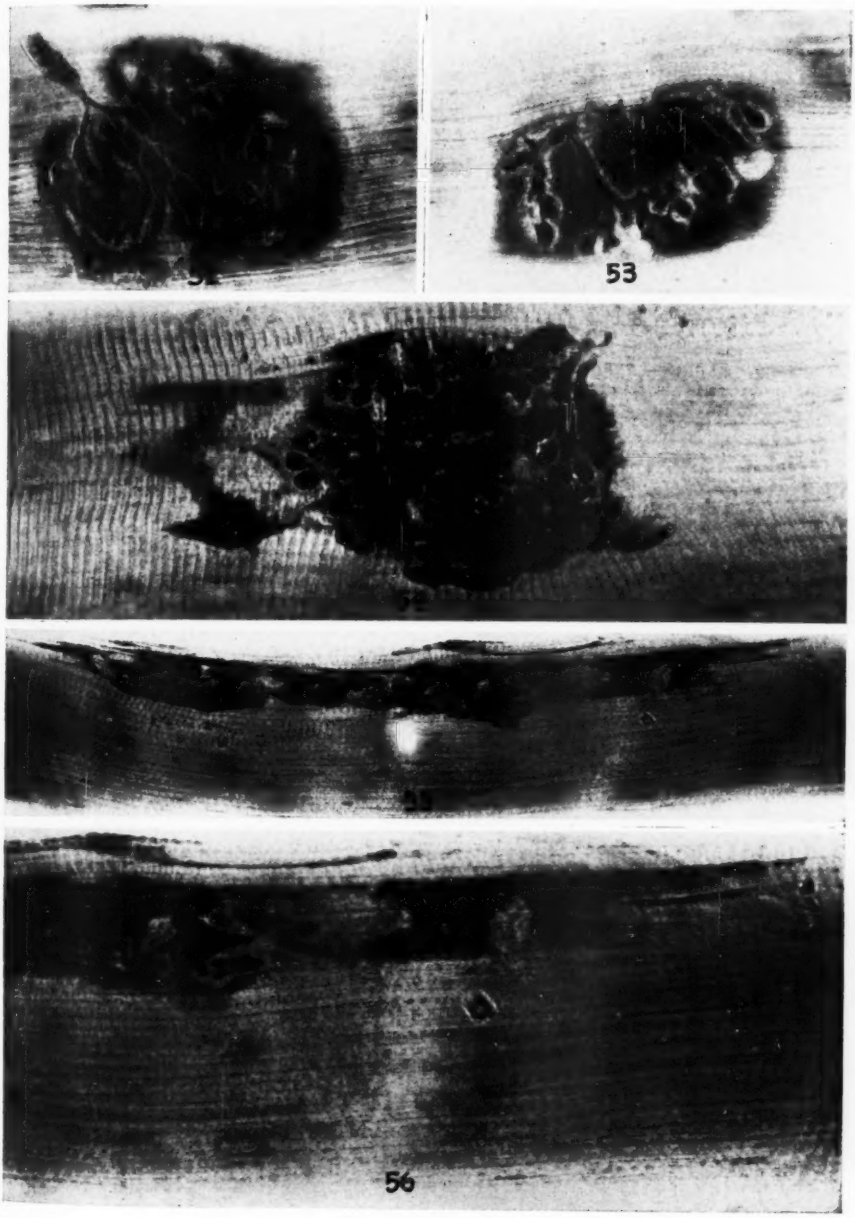


Carey

Motion and Secretion of Motor End-Plates

Plate 67

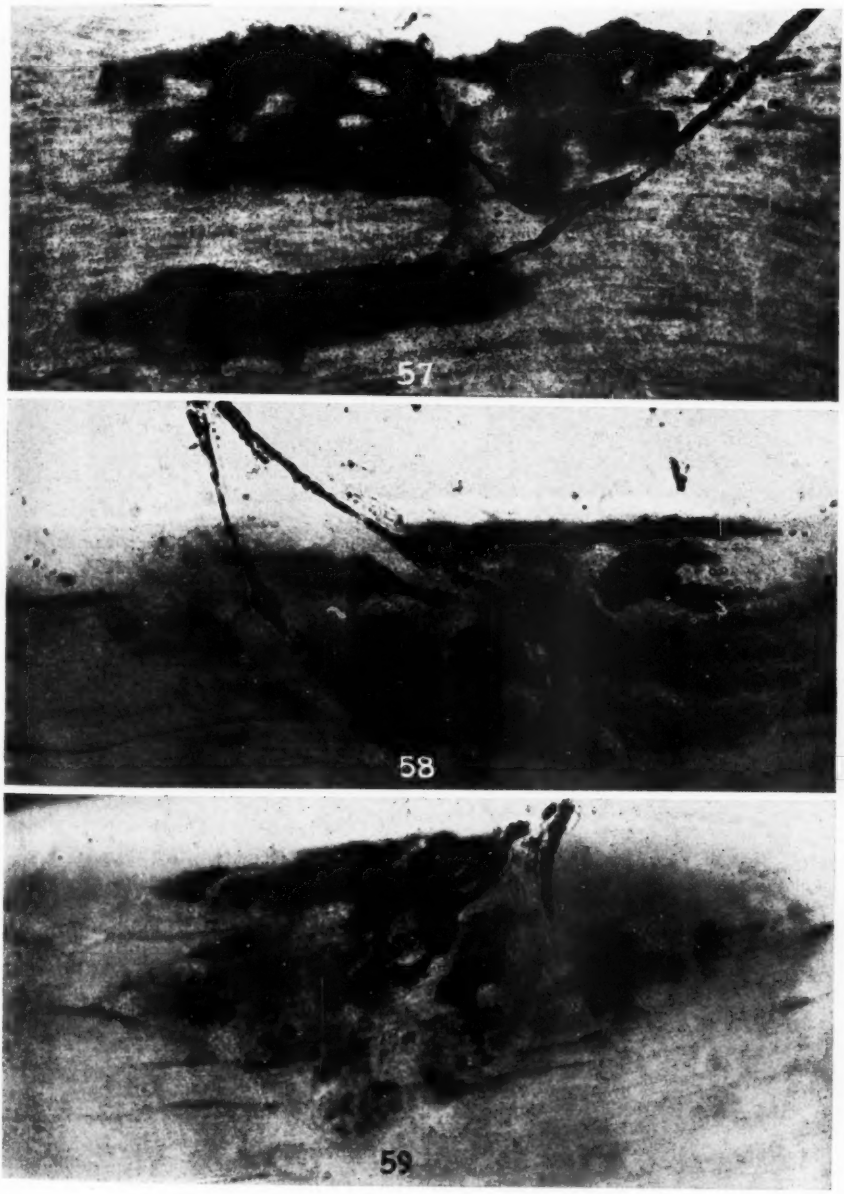
Figs. 52 to 56. Pleomorphism of motor end-plates in the biceps femoris muscle. Intocostrin was injected into the muscle locally and 1 minute later prostigmine was injected into the peritoneal cavity. There is an abnormal accumulation of the secreted granules of Kühne around the hypolemmal axons. Streamers of the secreted granules of Kühne which failed to disperse normally are produced by the chemical combination with curare (Figs 54 to 56). The gradual transformation of the globular terminals of the hypolemmal axons into Kühne's granules is evident in the right half of the illustrations (Figs. 55 and 56). There is a gradual transition from left to right of clear-cut hypolemmal axons surrounded by light spaces into the granular material of the elongated sole plate of Kühne without the intervening clear halo-like spaces. At the left (Figs. 55 and 56) the islands of concentrated Kühne's granules take an intense stain with gold comparable to that of the terminals of the axon. There is an antagonism between the stimulus of expansion produced by prostigmine and the stimulus of retraction produced by intocostrin which results in the clear-cut morphology of the hypolemmal axons and abnormal accumulation of the granules of Kühne. This is morphologic evidence that the terminal axons in the end-plate are microscopic endocrine glands. Their secretion motivates the muscle fibers by a transmitter substance—the granules of Kühne. A precipitate comparable to that of Kühne's granules is produced, *in vitro*, by the chemical interaction of either acetylcholine or choline with gold chloride. At the present time there is no good histologic test for either acetylcholine or choline at the myoneural junction except possibly that of gold chloride. Most other histologic methods destroy the granular sole plate of Kühne. The motor end-plates (Figs. 52 to 72) were obtained from the same biceps femoris muscle after the local injection of intocostrin into the muscle and prostigmine into the peritoneal cavity. (Fig. 55 magnified 300 ×; Figs. 52, 53, 54 and 56 are 750 ×).



Carey Motion and Secretion of Motor End-Plates

PLATE 68

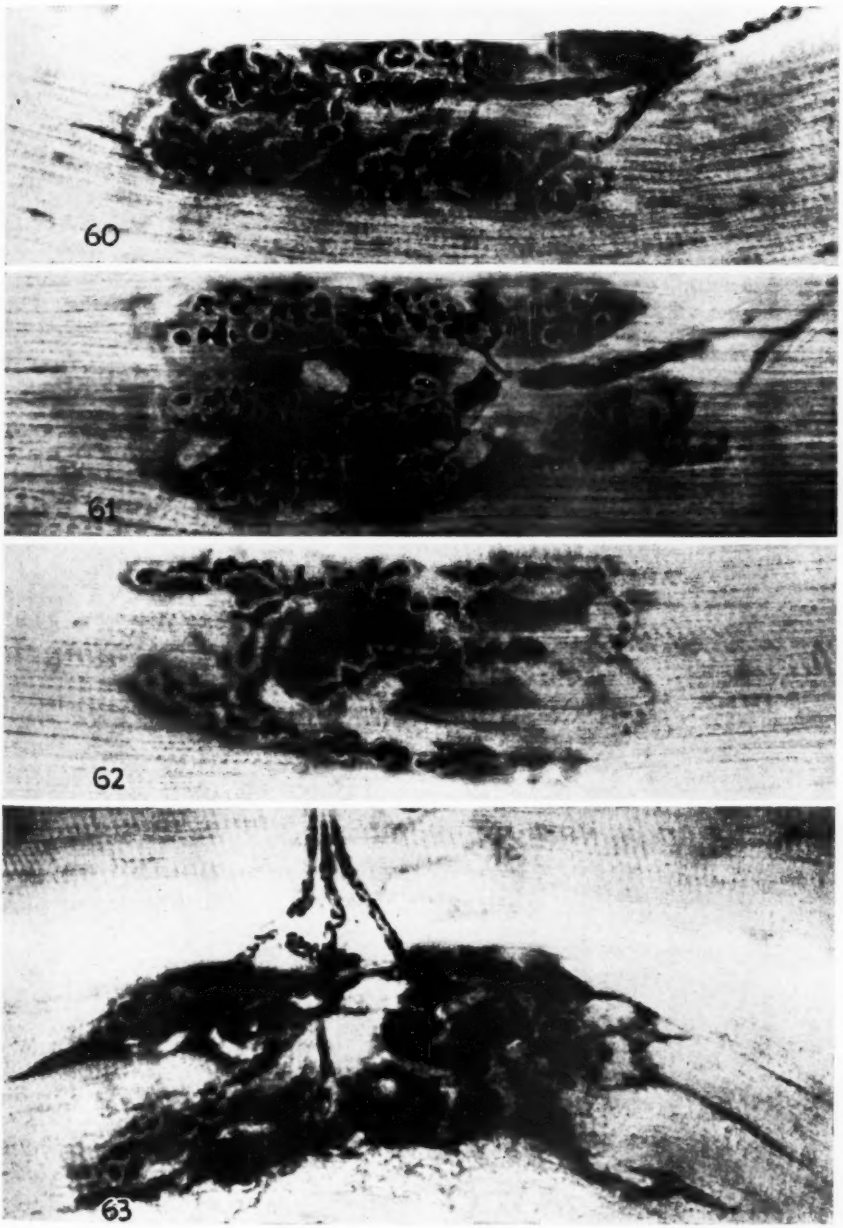
FIGS. 57 to 59. Greatly expanded motor end-plates in the biceps femoris muscle. This pleomorphism followed the injection into the muscle of intocostrin and later the injection of prostigmine into the peritoneal cavity. There is an abnormal accumulation of the granules of Kühne (Fig. 57) and a gradual transition of the hypolemmal axons (Figs. 58 and 59) into the granules of Kühne without the intervention of the clear halo-like space. There appears to be a direct transformation of the terminals of the hypolemmal axons into the secreted granules of Kühne through the violent chemical excitation produced by prostigmine and of inhibition by curare. There is a more gradual dispersion of the granules of Kühne into the substance of the cross-striated muscle fiber than that produced by the chemical action of either quinine or curare. The expansion effect on the motor end-plate produced by prostigmine appears to neutralize partially the microscopic changes in the motor end-plate produced by either curare or quinine acting alone. These antagonistic chemical actions give favorable evidence of the transformation of the axon into the specific secreted transmitter substance. The granular sole plate of Kühne, therefore, is not a constant, fixed and preformed structure. × 750.



Carey Motion and Secretion of Motor End-Plates

PLATE 69

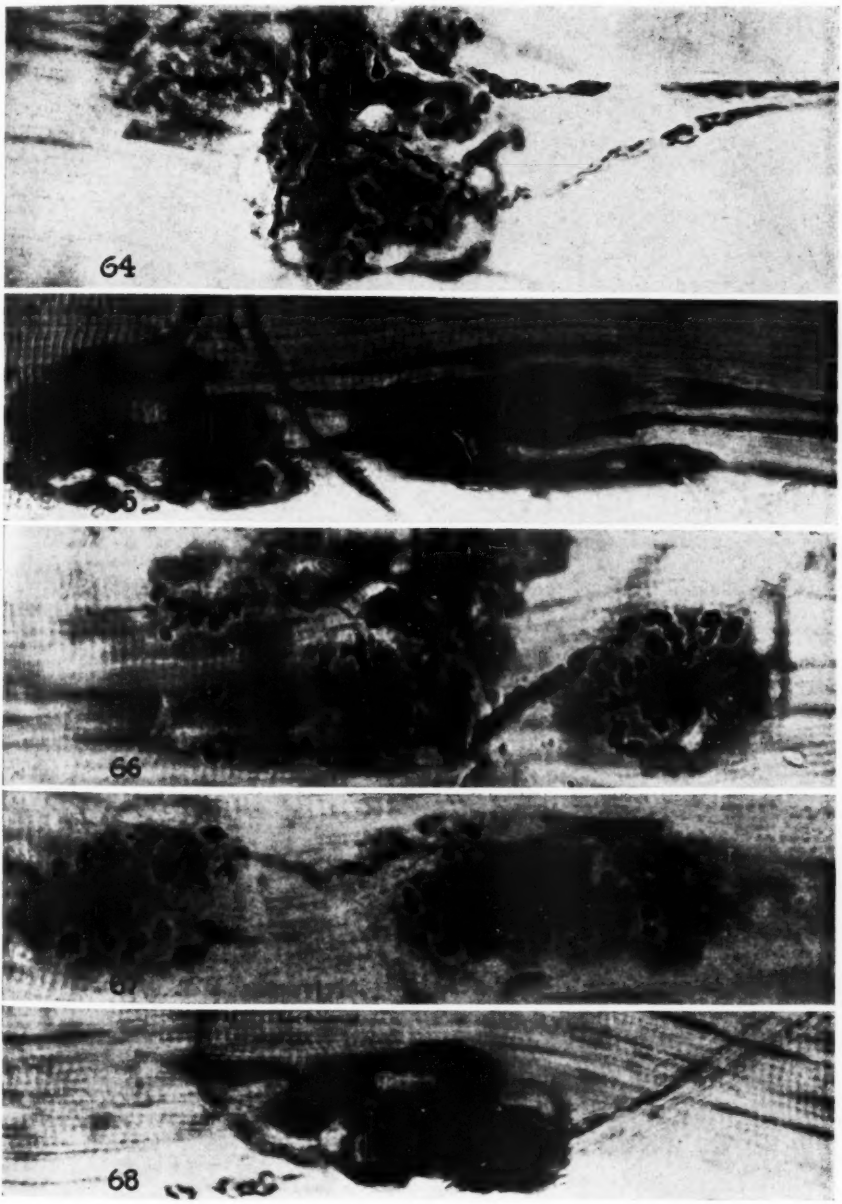
FIGS. 60 to 63. Pleomorphism by ameboid motion of the axonic branches in the motor end-plate in the biceps femoris muscle of the chameleon. Intocostrin was injected into the muscle locally and 1 minute later prostigmine was injected intraperitoneally. The chameleon died in violent spasm 2 minutes after prostigmine was injected. There is an accumulation of the granules of Kühne around the hypolemmal axons which have undergone, in many instances, globular fragmentation. These droplets vary from 4 to 15 μ in diameter. The clear oval spaces in the granules of Kühne are occupied by nuclei. This blocking of the dispersal of Kühne's granules by curare, followed relatively soon by chemical excitation with prostigmine, results in neurocladism or dichotomous division of the branches of the axon in the end-plate. This centrifugal extension and separation of the terminal branches in the end-plate and the simultaneous block to the dispersal of the granules of Kühne result in the abnormal accumulation of these granules around the related axon. The gradual replacement of the axon directly into the granules of Kühne is observed in the lower and right aspect of the end-plate in Figure 62. To the left and right of the end-plate (Fig. 63) there are elongated streamers of the granules of Kühne that have been agglutinated by the chemical retraction action of intocostrin and projection action of prostigmine. × 750.



Carey Motion and Secretion of Motor End-Plates

PLATE 70

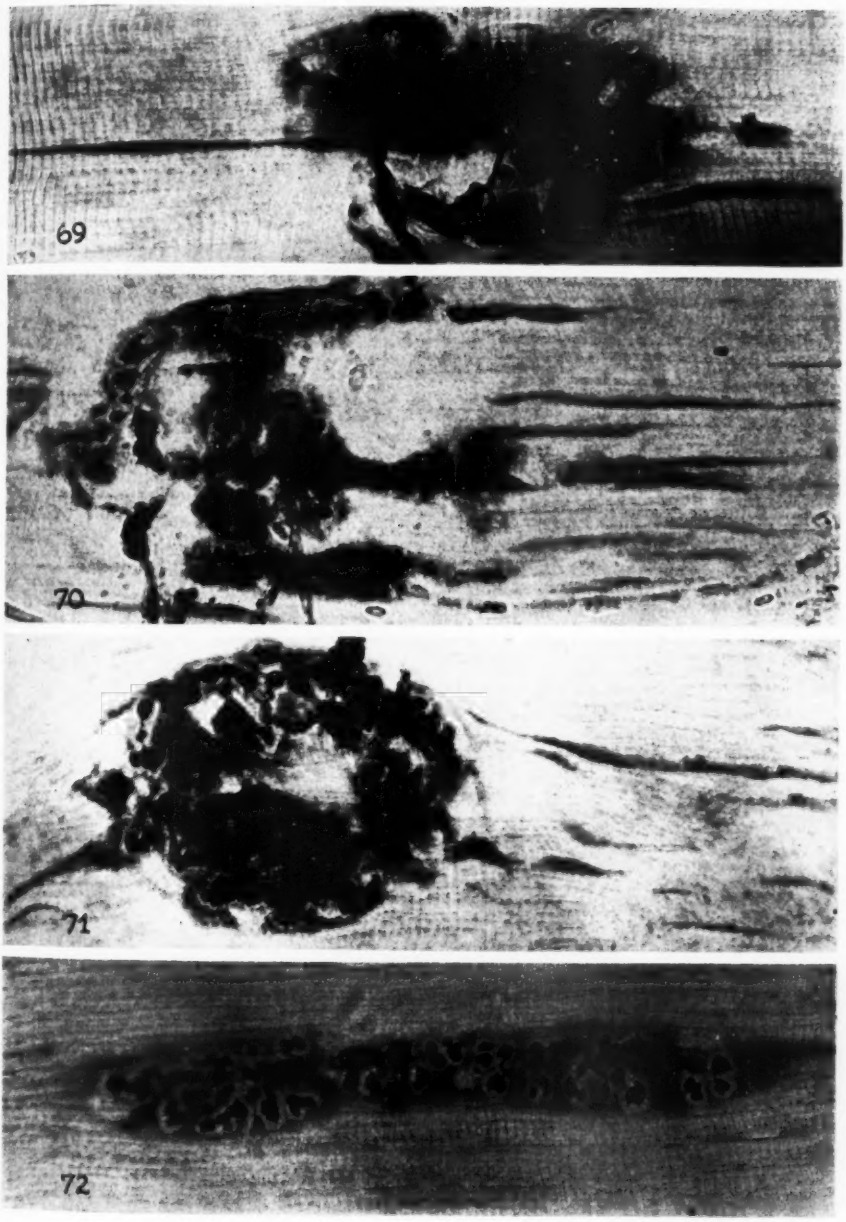
FIGS. 64 to 68. Various stages in the expansion and globular fragmentation of the hypolemmal axons and the accumulation of the surrounding granular sole plate of Kühne. Intocostrin was injected into the muscle locally and 1 minute later acetylcholine was injected intraperitoneally. The chameleons were decapitated in violent spasm 1 minute after acetylcholine was injected. The pleomorphic changes in these motor end-plates are the results of mutual antagonism of curare and acetylcholine. The curare partially blocks the transmission of the granules of Kühne into the muscle substance resulting in abnormal accumulations around the branches of the axons and agglutinated streamers of these granules in the muscle substance. The acetylcholine excites the expansive phase of the ameboid motion of the hypolemmal axons. The granules of Kühne (Figs. 65, 67 and 68) have a more intense affinity for the gold chloride than the dark bands of the cross striations in the muscle substance. × 750.



Carey Motion and Secretion of Motor End-Plates

PLATE 71

FIGS. 69 to 72. Pleomorphism of the hypolemmal axons of the motor end-plates produced by the chemical action of intocostrin followed by acetylcholine. Intocostrin was injected locally in the biceps femoris muscle of the chameleon and 1 minute later acetylcholine was injected in the intraperitoneal cavity. These end-plates were from the same muscle as those fibers illustrated in Plate 69. There is an abnormal accumulation of the granules of Kühne into insular masses found between the axonic branches as well as streamers of these granules projected away from the end-plate (Figs. 69 to 71). These granular streamers represent inadequate dispersal of the transmitter substance which is secreted from the hypolemmal axons into the muscle fiber. Globular fragmentation of the hypolemmal axon is clearly evident in the elongated end-plate (Fig. 72). × 750.

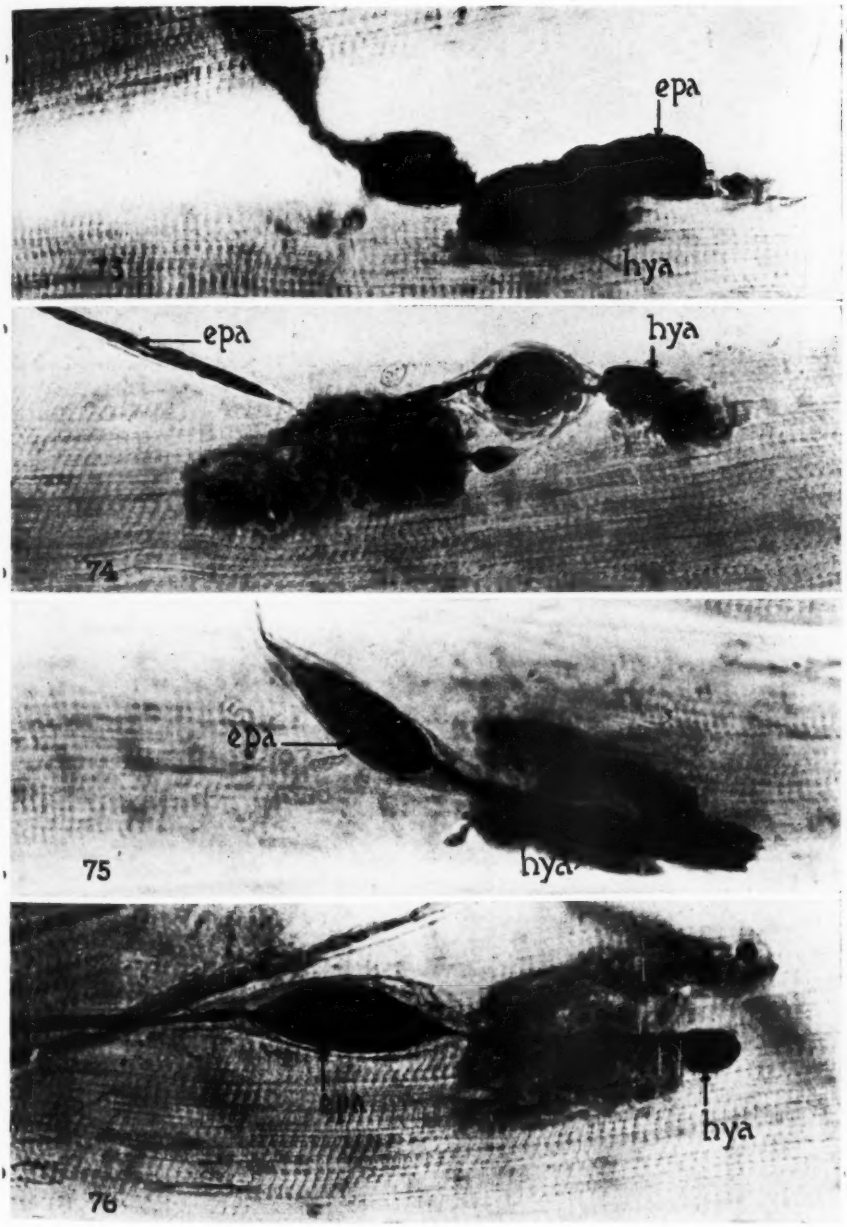


Carey

Motion and Secretion of Motor End-Plates

PLATE 72

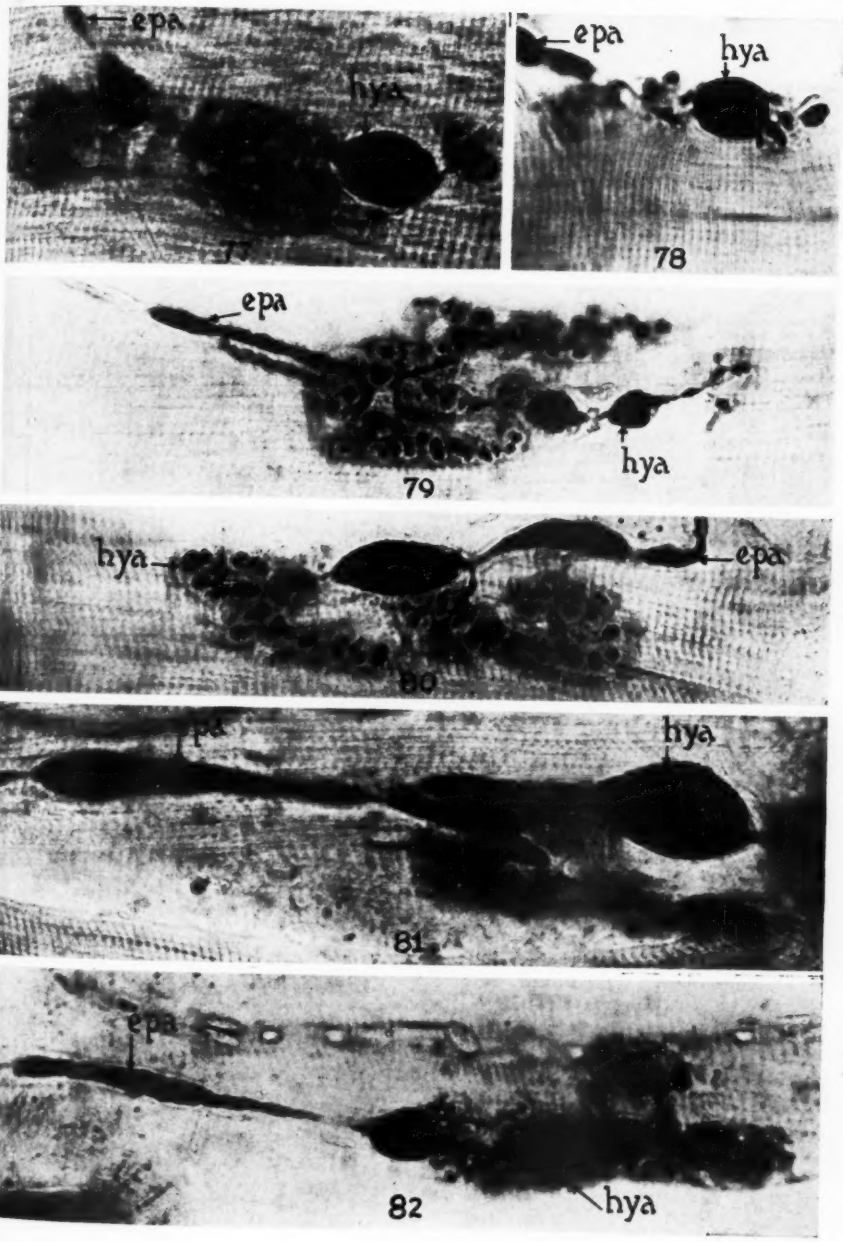
FIGS. 73 to 76. Pleomorphism of the epilemmal (epa.) and hypolemmal (hya.) axons of motor end-plates in the biceps femoris muscle of the chameleon. Intocostrin was injected locally into the biceps femoris muscle and 3 minutes later quinine sulfate was injected into the same location. Three minutes after the injection of the quinine sulfate locally, ammonium hydroxide was injected into the peritoneal cavity. The animal died in 2 minutes after injection of ammonium hydroxide in a state of violent spasm. One per cent of the end-plates had acute retention cysts which contained a substance that had strong affinity for gold chloride. These acute retention cysts were found either in the epilemmal (Fig. 73) or hypolemmal (Fig. 74) axons or they were found in both locations (Figs. 75 and 76). The morphologic accompaniment of these acute retention cysts which contained aurophilic substance was the diminution in the projection of the hypolemmal axons. The chemical block to the secretion of the granules by the hypolemmal axons together with excitation in the transmission of the nerve substance to the end-plate may be compared roughly to the production of a lake behind the dam erected in the course of a flowing river. This is experimental evidence that a liquid nerve substance is secreted normally from the motor end-plates into the muscle substance. Intocostrin and quinine apparently form a dense precipitation-membrane, by astringent action, around the periphery of the naked hypolemmal axons. This impermeable precipitation-membrane would then inhibit the normal transference of the axonic substance into the secretion granules of Kühne. Where this mechanical block by chemical action has become adequate, the circumscribed dilatations of the hypolemmal axons appear to possess a thickened membrane around which there is a diminution or complete absence of the granules of Kühne (Figs. 74 and 76). In other locations of the same end-plates, where the block has not become complete, there are hypolemmal axons surrounded by granules of Kühne. The teasing technic of muscle fibers impregnated previously with gold chloride preserves the anatomic continuity of the epilemmal axon, hypolemmal axon, ramifications of the terminal axons, the granules of Kühne and the muscle striations. × 750.



Carey Motion and Secretion of Motor End-Plates

PLATE 73

FIGS. 77 to 82. Pleomorphism of the epilemmal (epa.) and hypolemmal (hya.) axons of motor end-plates in the biceps femoris muscle of the chameleon. Intocostrin was injected locally into the biceps femoris muscle and 3 minutes later quinine sulfate was injected into the same location. Three minutes after the injection of the quinine sulfate locally, ammonium hydroxide was injected into the peritoneal cavity. The animal died in 2 minutes following injection of ammonium hydroxide in a state of violent spasm. One per cent of the end-plates had acute retention cysts which contained a substance that had strong affinity for gold chloride. These acute retention cysts were found either in the epilemmal (Figs. 80, 81 and 82) or hypolemmal axon or in both locations (Figs. 77, 78, 79, 80, 81 and 82). The morphologic accompaniment of these acute retention cysts which contained aurophilic substance was the diminution in the projection of the hypolemmal axons. These acute retention cysts are surrounded by a definite, circumscribed membrane which apparently has been thickened and precipitated by the chemical actions of intocostrin and quinine. There are no secretion granules of Kühne surrounding these cystic dilatations of the hypolemmal axon. Apparently an effective block has been produced to the secretion of Kühne's granules around the retention cysts which contain a substance having a very strong affinity for gold chloride. × 750.



Carey

Motion and Secretion of Motor End-Plates

PLATE 74

FIGS. 83 and 84. Sudden conduction of masses of nerve substance into the motor end-plates of the biceps femoris muscle of the chameleon by the action of tetraethyl lead injected into the peritoneal cavity. This acute conduction of nerve substance into the end-plate results in a complete distortion by the explosive action of this substance which disrupts the end-plates. There are radiations of gold-staining substance extending from the terminals of the distorted end-plates as though a violent explosion had destroyed the normal morphology of the end-plate. There is an abnormal accumulation of gold-staining substance in the disrupted plates. This likewise applies to the morphology of the cross-striated muscle substance in close proximity to these end-plates. × 300.

Carey

Motion and Secretion of Motor End-Plates

Plate 75

Figs. 85 to 87. Motor end-plates in the biceps femoris muscle of the chameleon disrupted by the explosive chemical action of tetraethyl lead injected into the peritoneal cavity. There is a massive conduction of an increased amount of gold-staining substance into the motor end-plates, which massive conduction appears to have a violent, explosive effect by destroying the normal morphology of the end-plate. Radiations of gold-staining substance extend from the terminals of the destroyed end-plate. These radiations appear to be the effect of explosive violence which radiates out into the striated muscle substance and destroys the normal morphology of the cross striations in many places. The chemical action of tetraethyl lead apparently delivers suddenly abnormal amounts of the gold-staining axonal substance to the end-plate. This supernormal transmission of nerve substance destroys the myoneural junction with explosive violence and shatters the normal morphology by a real intramuscular chemical explosion of some of the motor end-plates. × 750.

Carey

Motion and Secretion of Motor End-Plates

PULMONARY MUCOUS EPITHELIAL HYPERPLASIA (PULMONARY ADENOMATOSIS)

A Report of Two Cases *

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In the first article relative to pulmonary mucous epithelial hyperplasia (pulmonary adenomatosis) in man, Helly [1] remarked that it was a rare tumor which he described "to arouse interest because only the study of many similar cases will clear up the subject." In the past 4 years interest has again been aroused in this condition by the publication of reports of three similar cases by Bonne,[2] Richardson [3] and Sims.[4] In Sims' article, a most thorough and exhaustive review of the literature, the similarity of this condition to one seen in sheep was emphasized. This latter disease, variously known as jagsiekte, epizootic adenomatosis, and pulmonary adenomatosis, has a wide incidence in the sheep herds in South Africa, Iceland, Montana and Germany. Six human cases in all have been previously described.[1-6]

Report of Cases

Case 1

Clinical Course. The first case was that of a white male, 62 years of age, who was admitted to the Essex County Sanatorium on August 8, 1941, with a cough of 8 months' duration. About 1 month before developing his cough he began to notice undue fatigue. His cough became worse and during the winter of 1940-41 productive of 10 to 20 ounces of white, sticky, foamy sputum in 24 hours. In January, 1941, he began to notice loss of weight and strength. In March there was dyspnea on slight exertion. He consulted a doctor in June who sent him to the Sanatorium's Outpatient Department, after he had had to quit work. On July 20th he was advised to enter the Sanatorium.

His past history was negative except for "pleurisy" in the fall of 1940. One of his brothers was thought to have had tuberculosis in 1920.

He was a fairly well developed and well nourished man (height, 67¼ inches; weight, 145 pounds) who did not appear in acute distress. His temperature, pulse, respiration and blood pressure were not remarkable. Chest expansion was limited. There was no dullness to percussion and many coarse moist râles were heard over the chest on auscultation. Otherwise physical examination was negative.

The hemoglobin content of the patient's blood was 80 per cent (Sahli). The white cell count was 11,400 with 74 per cent polymorphonuclear leukocytes, 24 per cent lymphocytes and 2 per cent large monocytes. There seemed to be a marked increase in platelets in the smear. The sedimentation rate was not increased. The urine was not remarkable. The sputum showed no tubercle bacilli on three examinations and but few leukocytes.

* Received for publication, July 1, 1943.

Roentgenograms of the chest taken on July 24th and August 9th showed essentially similar findings (Fig. 1). They were read as follows: "There are diffuse mottled flocculent areas from apex to third rib on the right. There is a homogeneous shadow from the third to the fifth rib. This shadow covers the entire base, but is lighter toward the costophrenic angle. There is heavy infiltration on the left from the apex to the third rib. Below, the entire lung is obscured by a homogeneous shadow." This was interpreted as possibly a fungus infection or a tumor. On August 18th a Bucky plate was taken (Fig. 2) which did not reveal anything of further interest.

The patient's von Pirquet tuberculin test was very weakly positive. Bronchoscopy on August 21st revealed a profuse mucoid secretion in both major bronchi which apparently interfered with respiration. No localized lesion was found in the tracheobronchial tree. The secretion was interpreted as consistent with cardiac decompensation.

Three days prior to his death on the 19th hospital day the patient became markedly dyspneic and cyanotic and was placed in an oxygen tent with temporary relief. He became febrile at this time and remained so until his death on August 27th.

Autopsy Findings

At autopsy, 2 hours post-mortem, the body was that of a moderately well developed, somewhat thin white male. The dome of the diaphragm was at the 7th rib on the right and the 7th intercostal space on the left. There were numerous fresh fibrinous adhesions in both the right and left pleural cavities. The only other significantly abnormal findings were confined to the lungs.

The left lung weighed an estimated 900 gm. It was firm and consolidated. The pleura was covered with a thin coat of yellow fibrin. On section the upper lobe was gray-white with a glairy, mucoid appearance. On scraping with the section knife, a large amount of tenacious mucus was expressed from the cut surface. At the apex there was a gray-red, more granular zone, with several small areas of cavitation varying from 0.4 to 1.5 cm in diameter. The lower lobe presented the same glairy, mucoid appearance. The bronchi contained moderate amounts of mucoid material, and their mucosae were reddened and edematous. The pulmonary vessels were negative. The right lung weighed an estimated 850 gm. It showed almost complete consolidation of the entire lung with a few small peripheral zones of aeration. On section, it presented the same picture as the left lower lobe.

Microscopically, the lungs showed the only pathological changes of note. The alveoli were largely dilated. A few contained mucus, but for the most part they were filled with leukocytes and blood. The majority of the alveolar walls taken at random from various lobes of the lungs were covered by a simple columnar epithelium. In a few areas this epithelium showed a transition to a cuboidal type, but as a rule the change from the abnormal epithelium to the usual type was

abrupt, as if the alveoli were being invaded by the pathological cells. In some areas the columnar epithelium had formed small papillary processes.

The columnar cells were slender with pale cytoplasm and basally located nuclei. The cells were somewhat larger than the usual epithelial cells of the bronchial mucosa and were not ciliated. The nuclei occupied approximately one-sixth of the length of the cells and were for the most part round or oval with a pale network of chromatin. No mitoses were noted.

The alveolar walls were minimally thickened and contained an unusual number of lymphocytes. No abnormal epithelial cells were found in the lymphoid tissues of the lung. In one area there was marked increase in the fibrous connective tissue with loss of the usual architecture. The columnar epithelium in this area was arranged in small acinus-like structures.

The bronchiolar epithelium and that of the alveolar ducts did not seem to be affected by this process.

Case 2

Clinical Course. The second case was that of a white female, 79 years of age, who was admitted to the Boston City Hospital on the Fifth Medical Service on October 23, 1942. On a previous admission to the hospital in 1932 a diagnosis of primary anemia had been established. Since then she had had occasional injections of liver extract and had taken three liver capsules daily until about a month before her last admission. In the 2 months prior to admission the patient lost 20 pounds in weight. For 2 months she had severe anorexia and a dull pain in her epigastrium. She became pale and faintly yellow. She was bedridden for 1 month before entry. Her family history and past history were otherwise negative.

She was a markedly emaciated small woman (height, 56½ inches; weight at autopsy, 70 pounds) with lemon-tinted skin. Her tongue was pale and smooth with atrophic papillae. Her neck veins were distended, and her heart seemed to be enlarged to the left with systolic apical and aortic murmurs. Her lungs were clear to percussion and auscultation and no râles were heard. Her liver was just palpable. Her knee, ankle and abdominal reflexes could not be obtained.

Her red cell count was 860,000 with 4.5 gm. of hemoglobin per 100 cc. of blood. Her white cell count was 3,800 with 54 per cent neutrophilic leukocytes, 43 per cent lymphocytes, 1 per cent eosinophilic leukocytes, and 2 per cent basophilic leukocytes. A smear showed anisocytosis, hyperchromia, polychromasia, and basophilic stippling of the red cells. The platelets seemed to be decreased. The hematocrit reading was 12 mm. There were 1.2 per cent of reticulocytes. Examination of the urine and a Hinton test of the blood were negative. Her sputum was not examined. A roentgenogram taken on November 8th (Fig. 3) was interpreted as showing consolidation at the left base with focal pneumonia or encapsulated fluid in the region of the right middle lobe.

The patient was given three transfusions of 500 cc. each of citrated whole blood during the first week of her hospital stay. She also received daily injections of liver extract. Her reticulocyte count reached its maximum level of 13 per cent on the

5th hospital day. On the 14th hospital day the red cell count had increased to its maximum of 4,000,000. At this time her hematocrit reading was 42 mm.

On the 8th hospital day the patient developed a cough and consolidation of the left lower lobe was diagnosed. Signs and symptoms indicated increased infection, and the white blood cell count rose to 10,000. Three days prior to death the patient was stuporous and failing rapidly. Chemotherapy was not used. The patient died on the 27th hospital day.

Autopsy Findings

At autopsy, 7 hours post-mortem, the body was that of a well developed, emaciated white woman. Her abdominal panniculus was less than 0.5 cm. in thickness. There were about 300 cc. of clear yellow fluid in the right pleural cavity. The posterior portion of the left pleural cavity was completely obliterated by firm fibrous adhesions so that the lower lobe had to be cut away from the parietal pleura by sharp dissection. The heart weighed 280 gm. and was not remarkable. The gastric mucosa was markedly atrophic. Many diverticula were found in the sigmoid portion of the colon. These showed no surrounding reaction. The liver was small, weighing but 700 gm. It was somewhat browner than usual and cut with slightly increased resistance. It was not otherwise remarkable. The gallbladder contained a number of faceted stones, but its mucosa did not appear abnormal. The spleen weighed 120 gm. and was not remarkable. The kidneys weighed 190 gm. They showed a coarsely granular surface and, on section, a moderately thinned cortex which was well demarcated from the medulla. The bladder was dilated and contained 750 cc. of clear amber urine. The genital organs were markedly atrophic. There was moderate atheromatosis of the abdominal portion of the aorta. The lumbar, sternal, costal and femoral bone marrow was markedly soft, dark red and gelatinous.

The most significant findings were those in the lungs. The right weighed 700 gm. The lower and middle lobes were consolidated and showed no crepitation. The upper lobe was subcrepitant. On section the lower and middle lobes revealed a smooth cut surface which was dark red peripherally and gray in the central portions. The lower portion of the upper lobe was similar to the peripheral portion of the lower lobe. The rest of the upper lobe was dark red and yielded serosanguinous fluid on pressure. The consolidated portions of the lung yielded sanguinopurulent scrapings.

While the left lung was being removed, the upper and lower lobes were inadvertently separated. The lung weighed 420 gm. The lower lobe was ovoid and measured 8 by 6 by 4 cm. On section it was firm and pale gray, and had a gelatinous pink-gray, mulberry-like tumor process involving the central portion of the lobe. The bronchi were

small but unusually prominent. The left upper lobe resembled the right upper lobe except that the lingula contained several small spherical areas resembling the pink-gray gelatinous area described in the lower lobe. The trachea and bronchi of both lungs were congested and contained a moderate amount of mucoid secretion. The pulmonary vessels were not remarkable.

Culture of the heart's blood and of the right lower lobe revealed *Diplococcus pneumoniae,* type VIII. Culture of the left lower lobe revealed *D. pneumoniae,* type VIII, and hemolytic *Staphylococcus aureus.* A Ziehl-Neelsen stain of the lung revealed no acid-fast organisms.

On microscopical examination, the arterioles in most of the viscera showed a moderate amount of hyaline change. The perinuclear yellow pigment of the heart muscle cells was somewhat more prominent than usual. There were no changes of note in the liver. The kidneys showed occasional fibrosed glomeruli. No parietal (eosinophilic) cells could be found in the mucosa of the stomach. The bone marrow showed moderately active hematopoiesis as is seen in adequately treated primary anemias.

The lung sections showed the microscopical changes which were of interest. The majority of the sections showed merely a confluent bronchopneumonia. The unusual findings were confined to the left lower lobe, to the lingula of the left upper lobe, and to a small portion of the right upper lobe.

In scattered nodules in the upper lobes, the alveoli were lined with columnar epithelium. The epithelial cells were similar to those of the first case except that they were proportionately higher so that they almost obliterated the lumen of the alveoli in some areas. The surrounding alveoli were filled with mucoid secretion and desquamated cells, apparently of epithelial origin. Aniline blue stains revealed that the cells as well were filled with mucus. Cuboidal epithelium was not seen and no bronchioles could be identified with certainty. There was no invasion of the lymphoid tissues of the lungs.

The left lower lobe had been almost completely replaced by hyalinized collagenous fibrous tissue. There were islands of abnormal epithelium lining spaces as large as bronchioles. Areas of alveoli lined with abnormal epithelium also were seen. The bronchi and bronchioles appeared to be unaffected.

Elastic tissue stains revealed no change and aniline blue stains revealed a slight increase in the fibrous tissue of the alveolar septa in the areas involved by the process in question.

Discussion

The pathological picture as described in the two cases reported is essentially similar to that described in earlier articles. In both cases the disease picture was somewhat obscured by severe superimposed bacterial infections. The gross picture in this condition is very similar to that seen in Friedlander's pneumonia. Even microscopically the condition might easily be overlooked in cases with severe bacterial infections, so that it is not impossible that other cases of this nature have not been noticed in the past.

In the first case the clinical course can readily be correlated with the pathological findings, as in Helly's [1] case. The clinical course in both was similar to that of pulmonary tuberculosis. In the second case, however, the mucous epithelial hyperplasia was an incidental finding at autopsy which was not recognized until the microscopical sections were seen. The roentgenographic pictures in both cases were similar and an infectious process or a tumor was suspected in each.

The duration of the disease in these cases is problematical. In both of them there were areas of marked fibrosis in the lungs which seemed to indicate a protracted, chronic infection rather than an acute or neoplastic process. The most acute case in the literature, the second described by Oberndorfer,[6] was accompanied by an acute hemorrhagic pneumonitis of short duration in a 21-year-old male. In sheep, on the other hand, the course is not as acute. Sheep die, as a rule, within a few months after the infection is first noted, although some may live for more than a year.[7]

In the discussions of this and similar diseases in the literature, emphasis has been placed on the importance of such lesions in the consideration of the genesis of carcinomas of the lung. There are several phases of this problem upon which it is worth while to speculate. In the first place, where do these proliferating cells arise? Helly [1] favored the epithelium of the alveolar ducts for several reasons: (1) because the cells were nonciliated in contrast to those of the bronchioles; (2) because the cells could be seen extending from the alveoli to the bronchiolar mucosa but no farther; and (3) because there was a sudden transition from the abnormal cells to the usual alveolar lining. Oberndorfer,[6] on the other hand, was of the opinion that in his case, at least, the tumor arose from the lining cells of the alveoli which he believed have an epithelial origin. He felt that he could demonstrate gradual transitions between pathological cells and the usual alveolar lining cells.

In our opinion, a definite decision is difficult to arrive at; but because of their apparently peripheral and multicentric origin, it seems not unlikely that the abnormal cells arise from the alveolar lining cells (also the opinion of Dr. J. L. Bremer*), and provide further evidence for the epithelial nature of those cells. The changes are of a hyperplastic nature and there is also some metaplasia; for, although the cells are not ciliated, they are columnar and produce large quantities of mucus. In support of our contention, we note that this picture in no way resembles that seen in pulmonary adenomas of which the structure has been quite definitely established in recent years.[8] In that condition the hyperplastic nodules have ill-defined borders and are not encapsulated. They have no stroma other than that of the alveolar walls. Their cells frequently fail to fill more than a small portion of an alveolus. Their exact site of origin is somewhat more problematical.

It seems to us that Oberndorfer's [6] ideas are quite in accord with the picture presented in cases of the type which we have described. Helly's [1] ideas cannot be dismissed readily, however. In one of our cases intact alveolar duct epithelium was seen in several areas in which the alveoli were completely lined with abnormal cells. Thus in this case the microscopical picture is at variance with Helly's opinion. In sheep there are peculiar mucous glands in the walls of the respiratory bronchioles, and many authors believe that it is there that the proliferation starts. From the nature of these cells in man it is not impossible that they may arise from the occasional mucous goblet cells which occur in the mucosa of the bronchioles.

Are these tumor masses of infectious origin? There has been but one case in the literature in which the nature of this disease has been suspected at the autopsy table and confirmed by frozen section.[3] In that case attempts were made to produce a similar condition in experimental animals by the usual methods with no success. That the microscopically similar disease in sheep is of an infectious nature is undoubtedly true, but transmission of the disease from sheep to sheep or to any other laboratory animal has been almost universally unsuccessful. However, the disease has been proved to be infectious in nature, as is evident from a study of the epidemic which occurred in Iceland.[9] It seems that a virus is the most tenable cause for the hyperplasia, since no bacterial species has been recovered with any regularity from affected sheep.

A number of human cases with a more or less similar microscopical

* Personal communication.

picture have been described in which, however, metastases have been found—usually to the regional lymph nodes and occasionally to bone marrow and the brain. References to 10 or 11 such cases have come to our attention.[10-17] The published descriptions of all but 3 of these [15-17] have been examined by us. They all present a somewhat similar picture to the apparently nonmalignant cases described by other authors, although the production of mucus is not a constant finding in the malignant cases as in the nonmalignant condition.

Thus in respect to these few instances, on a histological basis and by analogy, it can be said that these carcinomas may be of an infectious origin. Aynaud is said to have seen metastases in one case of jagsiekte in a sheep (cited by Dungal [9]). However, in our opinion these tumors have but little significance in the consideration of the origin of carcinomas of the lung in general, because the large majority of pulmonary carcinomas obviously do not arise in such a manner but are bronchiogenic.

In a recent review of the case reports of such carcinomas, Neubuerger and Geever [18] agreed that this type of tumor was rare, with an incidence of less than 5 per cent of all carcinomas of the lungs. They feel, as we do, that mucous epithelial hyperplasia may not be as rare as the number of reports in the literature would indicate.

Summary

Two cases of pulmonary mucous epithelial hyperplasia (pulmonary adenomatosis) are described. After reviewing the available literature, the possibility of a viral etiology is considered. While a definite decision cannot be made, it seems probable that the abnormal cells arise from the alveolar lining. Origin from the goblet cells of bronchiolar mucosa cannot be excluded. This condition is of but little significance in a consideration of the genesis of pulmonary carcinoma in general.

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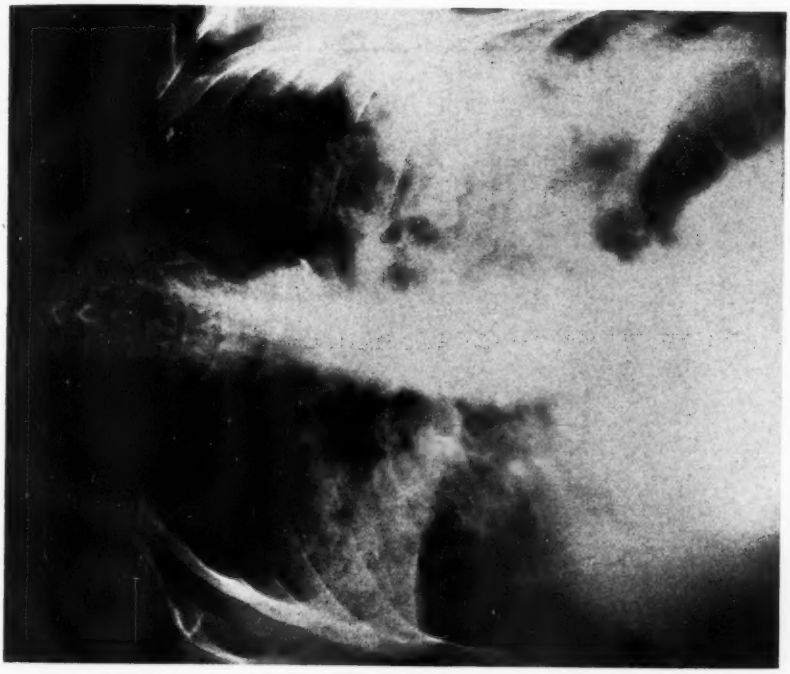
[*Illustrations follow*]

DESCRIPTION OF PLATES

PLATE 76

FIG. 1. Case 1. Roentgenogram taken on July 24, 1941.

FIG. 2. Case 1. Bucky plate roentgenogram taken on August 18, 1941.



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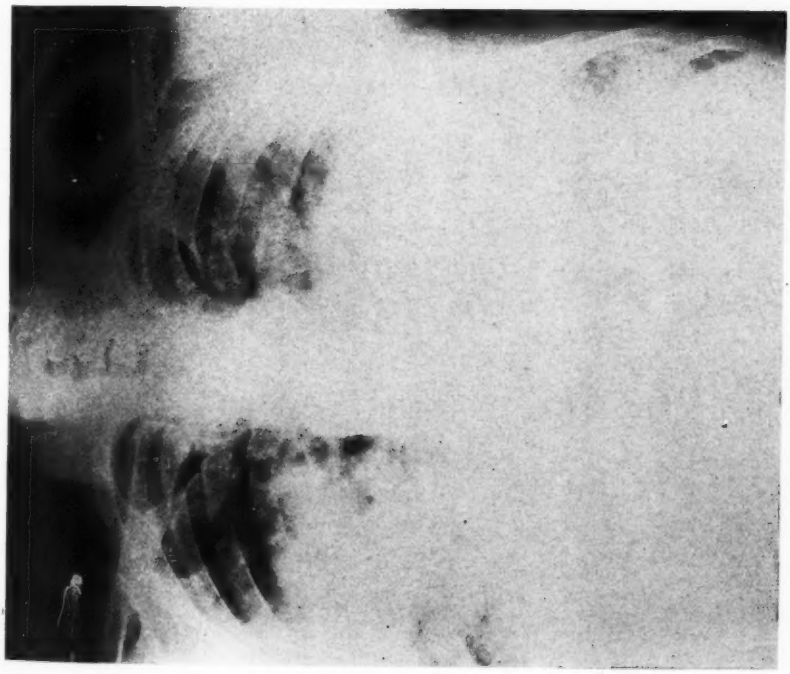

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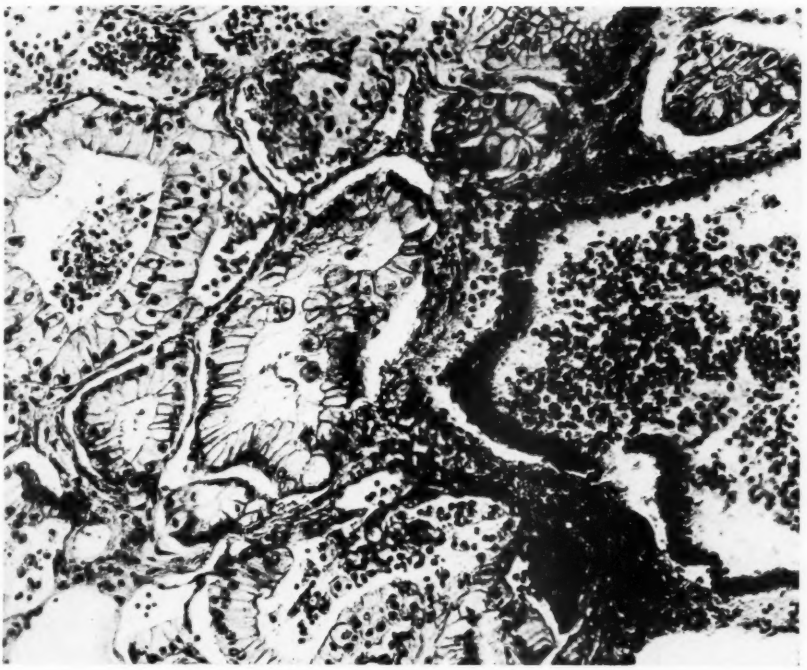
PLATE 77

FIG. 3. Case 2. Portable roentgenogram taken on November 18, 1942.

FIG. 4. Case 1. An uninvolved bronchiole with attached alveolar duct in an area with marked replacement of the usual alveolar lining by typical columnar, nonciliated epithelial cells. Hematoxylin and eosin stain. × 175.



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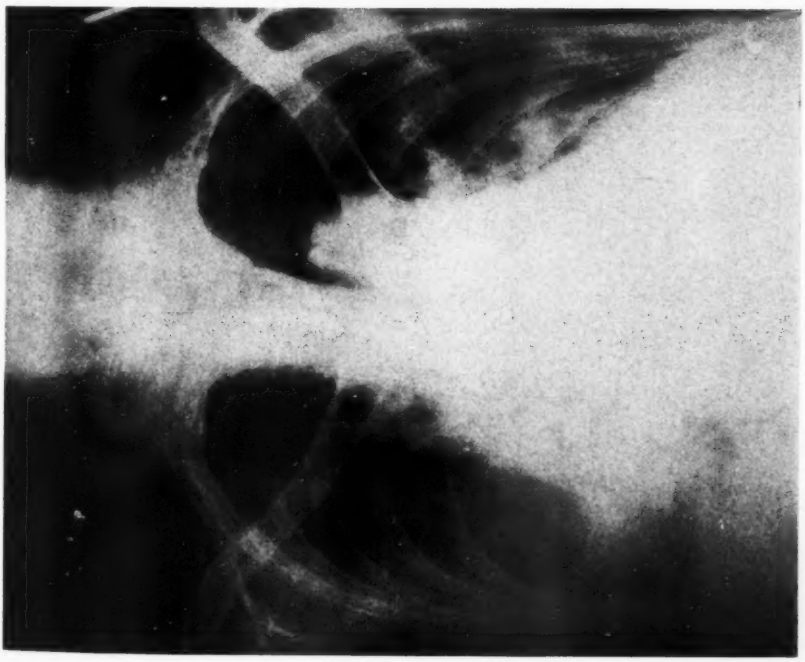

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Taft and Nickerson Pulmonary Mucous Epithelial Hyperplasia

PLATE 78

FIG. 5. Case 1. A small cluster of abnormally lined alveoli in the midst of uninvolved alveoli. Hematoxylin and eosin stain. × 175.

FIG. 6. Case 2. A small collection of abnormal alveoli with the surrounding spaces filled with large quantities of intensely stained mucus which contains desquamated cells. Phloxine and methylene blue stain. × 175.

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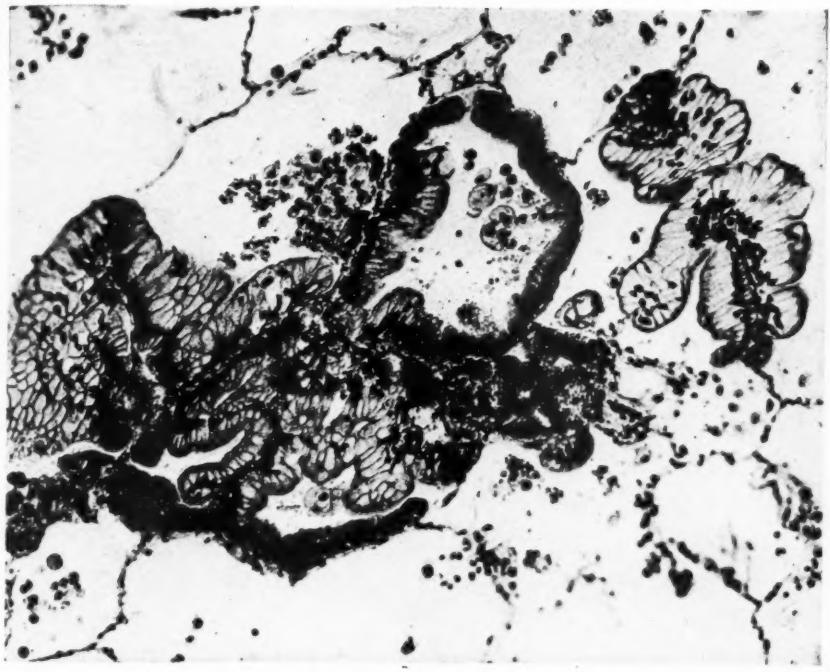

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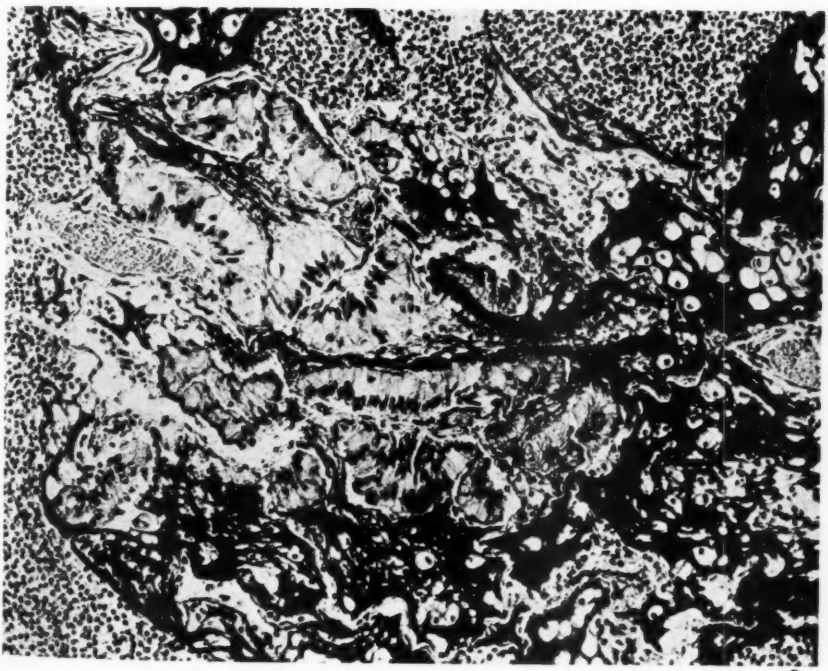

Taft and Nickerson Pulmonary Mucous Epithelial Hyperplasia

PLATE 79

FIG. 7. Case 2. Detail of the epithelial cells and alveolar walls to show that the latter are not involved or markedly changed by the hyperplastic process. Iron hematoxylin stain and Lee-Brown's modification of Mallory's aniline blue stain. × 390.

FIG. 8. Case 2. Photomicrograph of the left lower lobe, showing the marked fibrosis which the lobe has undergone. There is a central space lined with abnormal epithelium which shows moderately large papillary processes. Phloxine and methylene blue stain. × 175.

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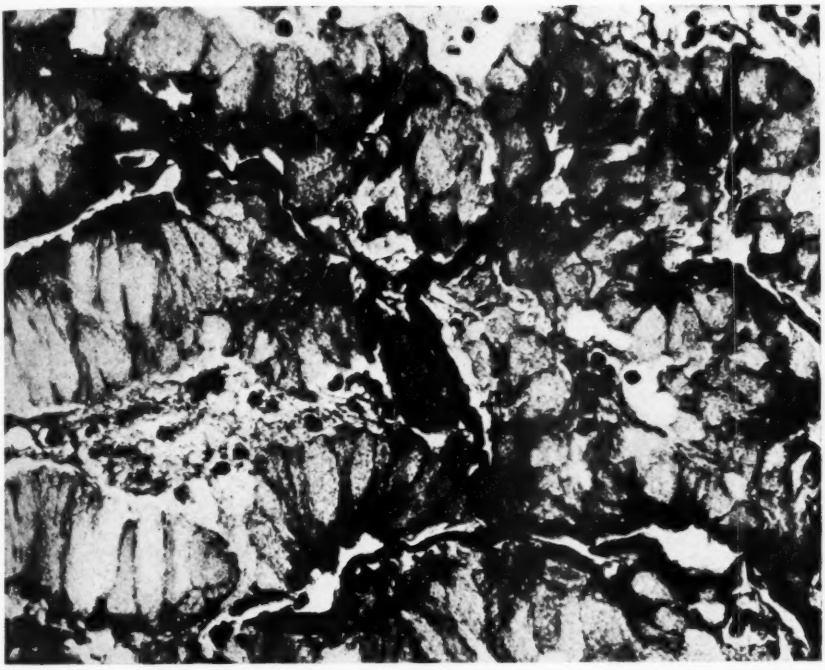

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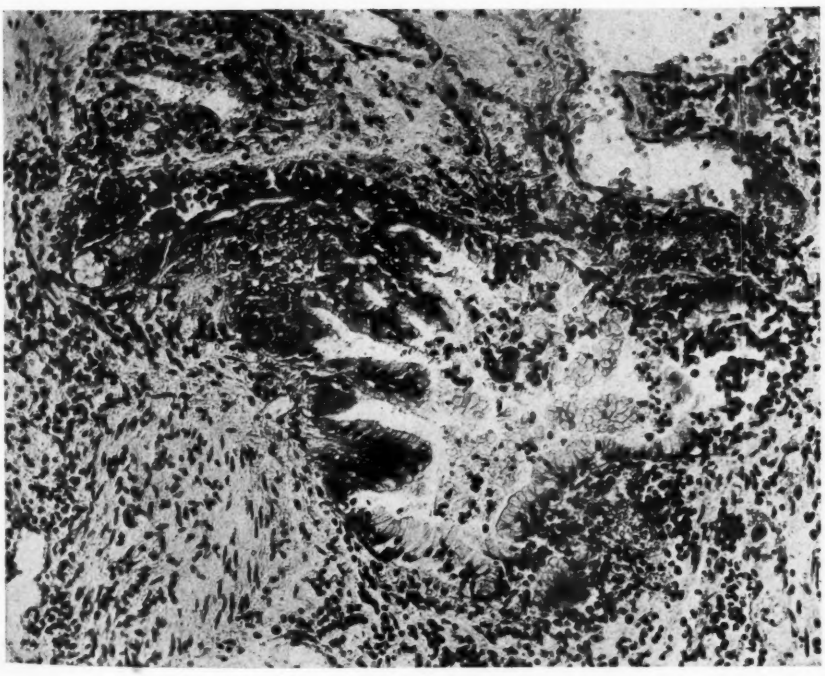

Taft and Nickerson Pulmonary Mucous Epithelial Hyperplasia

NEURILEMOMAS IN A FAMILY OF BROOK TROUT*

GEORGE A. YOUNG, JR., D.V.M., and PETER OLAFSON, D.V.M.
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The tumors described in this article were discovered during some bacteriological and pathological studies of trout which were being made to determine the cause of mild losses in the brood stock of a hatchery. Sections were made from all of the internal organs which were cultured for bacteria. Examination of these sections disclosed the presence of a characteristic tumor affecting the peripheral nervous system.

Tumors in fish are not uncommon and nearly every type of tumor reported in mammals occurs in fish. A report of a neoplasm of the peripheral nerves in trout, however, was not found in the literature.

MATERIAL

The material for this study was obtained from 41 trout. All but 7 of these were supplied by a single hatchery. The distribution of the species studied and the source of supply is indicated in Table 1.

The oldest trout obtained from hatchery no. 1 were known to have been inbred for at least 2 generations and the youngest fish were inbred from 3 to 4 generations. The extent of the inbreeding in the brood stock before it was placed in this hatchery is not known.

All tissues were fixed in formaldehyde, embedded in paraffin and stained with hematoxylin and eosin. Representative sections were stained by Masson's trichrome and Mallory's acid fuchsin technics. Gram's stain and an acid-fast stain were used in an attempt to demonstrate bacteria in a few selected sections. Because of the difficulty involved in handling the tiny organs of the young fish, only cross sections

TABLE I
Source of Material for Study

Species	Source and number of trout		
	Hatchery no. 1	Hatchery no. 2	Natural
Brook trout (*Salvelinus fontinalis*)	23	2	0
Brown trout (*Salmo trutta fario*)	7	1	0
Rainbow trout (*Salmo gairdnerii irideus*)	4	1	0
Lake trout (*Cristivomer namaycush*)	0	1	2

* Received for publication, June 7, 1943.

through the intestine with the adjoining pancreas and through the kidneys were made. Cross sections of the entire fish were made in studies of 1 and 2-month-old trout.

Incidence

All of the brook trout examined were found to be affected with neurilemomas. This group consisted of 25 trout obtained from two hatcheries. The severity of the affliction was found to be directly related to age, the organs of the oldest fish being most extensively involved. Both sexes were equally affected.

Studies were attempted on embryos and fry but the methods used in fixation made the yolk material too hard to section. Fingerlings which had just begun to feed were studied, however, and showed mild involvement of the nerves at an age of 1 month. Material was collected from July to January but no seasonal variation was evident. Two mature brook trout from hatchery no. 2 were found to have these tumors but to a lesser degree than trout of the same age from hatchery no. 1.

Only one trout of a different species was found to be affected with neurilemomas. This was a 2-year-old brown trout taken from hatchery no. 1. A single nerve trunk in this fish was found to be tumorous. All other brown trout were found to be negative. No rainbow or lake trout were affected.

Pathology

The pathological changes in this disease were principally microscopical. In some specimens moderate enlargement of the nerve trunks supplying the stomach, intestine and kidneys was seen. There were no other lesions except small surface ulcers in some specimens. There was no evidence to indicate any relationship between these ulcers and the tumors of nerves.

The tumors studied were uniform and characteristic in their structure and not unlike the neurilemomas described by Bailey and Herrmann.[1] All lesions were arranged more or less in whorls. The outermost portion consisted of a band of collagenous tissue of variable thickness surrounding concentric layers of fibrous tissue. These two tissue arrangements were constant. The centers of the lesions, however, were variable.

The most uniform lesions were made up of collagenous and fibrous concentric rings surrounding a group of cells. These cells were for the most part polyhedral with a variable amount of eosinophilic and finely

granular cytoplasm. The nuclear chromatin was scattered in many large sharply stained granules. This type of lesion is illustrated in Figure 4.

Other tumors consisted of laminated layers surrounding a mass of hyaline material (Fig. 3). Some of these lesions were calcified as illustrated in Figures 2 and 5. Large giant cells (Fig. 6) and brownish pigment were observed in some of the neurilemomatous nerve trunks.

The most extensive lesions occurred in the mesentery and pancreas. Many whorled masses surrounded and connected by collagen were observed. Figure 3 shows a representative area from a cross section of a tumorous nerve running through the mesentery in a 3-year-old brook trout. This nerve trunk was approximately 0.5 cm. in diameter. In fingerlings and yearlings, the lesions were generally limited to a few nerve trunks. Tumors in the very young fish, less than 2 months old, were limited to one or two foci in a cross section of the whole fish. The submucous and myenteric plexuses of the intestine were occasionally affected.

Involvement of the nerves in the kidneys was sometimes extensive. In one 4-year-old brook trout more than half of the kidney tissue in the section had been replaced by the affected nerve trunks. Renal involvement seemed to increase with age.

Nerves to other organs were found to be involved but not with the regularity of those in the mesentery and kidneys. Lesions were found occasionally in the nerves to the heart, testicle and spleen. In no case was the liver found to be involved.

In a limited study of the sensory nerves of the affected brook trout, all were found to be normal. The large lateral subcutaneous nerve was not found to be affected.

Inclusion bodies were not observed in any of the tissues. Many of the sections were examined with a micropolarizer but failed to show evidence of crystals. Representative tissue sections were stained with Gram's and acid-fast stains but no evidence of bacteria was found in any of the sections. Attempts to culture microorganisms from the liver, heart and kidneys of many of the fish were unsuccessful.

Discussion

The tumors in trout arose from the perineurium and endoneurium. Following the terminology suggested by Stout,[2] these tumors are called neurilemomas. There is considerable controversy as to the origin of tumors of this type but from the observations made in this study, the

connective tissue-like elements in and around the nerve fibers were primarily involved.

Tumors of the peripheral nerves have been reported in various mammals but the incidence has been low. This is in contrast to the very high incidence found in this family of brook trout.

The fact that every brook trout examined, regardless of age, was affected with a neurilemoma suggested that the condition is hereditary. Only one fish of related species was found to be affected. This trout was taken from the same hatchery as the affected brook trout. It is possible that cross-insemination may have occurred.

Studies of the affected nerves in brook trout indicated that the autonomic nerves were primarily involved. That both the sympathetic and parasympathetic nerves were affected was shown by involvement of the submucous and myenteric plexuses of the intestine. Nerves to the mesentery, heart and kidneys were also involved.

Summary

Twenty-five brook trout (*Salvelinus fontinalis*) were found to be 100 per cent affected with neurilemomas. All but 2 of these trout were obtained from a single hatchery. Only 1 of 16 fish of three other species was found to be affected. The high incidence in brook trout of all ages in this hatchery suggested that the condition may be hereditary. The autonomic nervous system was found to be primarily involved.

We wish to thank A. V. Tunison of the Cortland, N. Y., Experimental Hatchery for supplying the material for study and for information relating to trout.

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Description of Plates

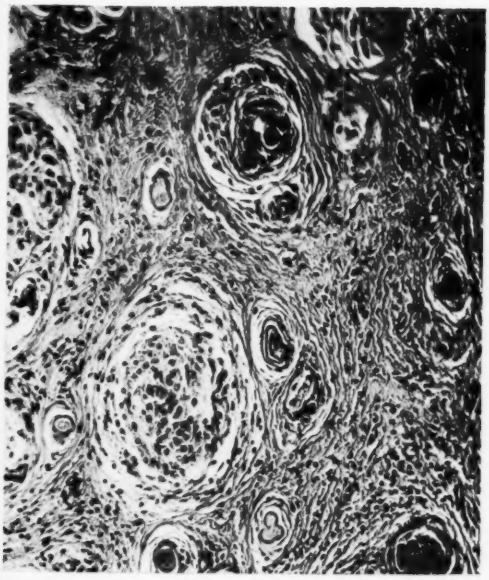
Plate 80

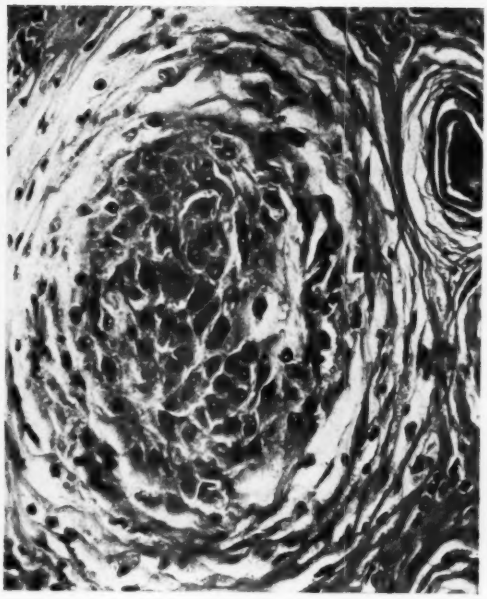
Fig. 1. Photograph of the stomach of a 3-year-old brook trout with an enlarged vagus nerve shown underneath the window cut in the organ.

Fig. 2. Section from the vagus nerve shown in Figure 1. Whorls and calcified whorls are shown. Hematoxylin and eosin stain.

Fig. 3. Section of a mesenteric nerve from a 3-year-old brook trout. Masson's trichrome stain.

Fig. 4. Enlargement of an area in Figure 3 showing polyhedral cells with darkly stained chromatin granules in the nuclei.





Young and Olafson

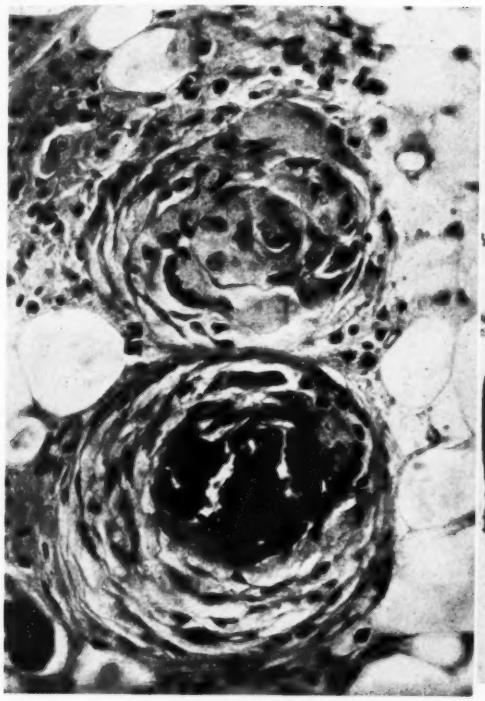
Neurilemomas in Brook Trout

PLATE 81

FIG. 5. Section of a nerve from the mesentery of a yearling brook trout showing a calcified whorl in the lower portion of the photomicrograph. A cross section of an axon is evident in the upper left-hand corner. Hematoxylin and eosin stain.

FIG. 6. Section of a cardiac nerve in a 2-year-old trout. A typical giant cell is shown. Hematoxylin and eosin stain.

FIG. 7. Section of the intestine in a 3-year-old brook trout with involvement of the submucous plexus. Masson's trichrome stain.



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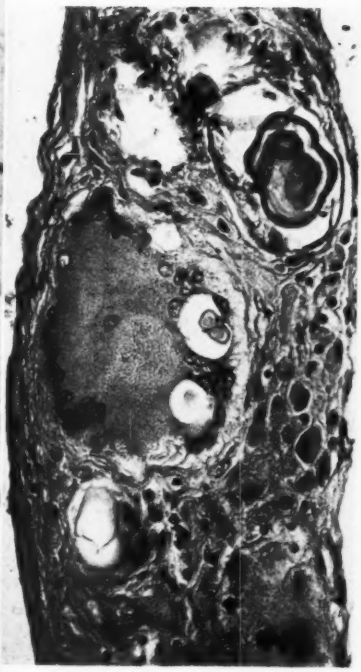

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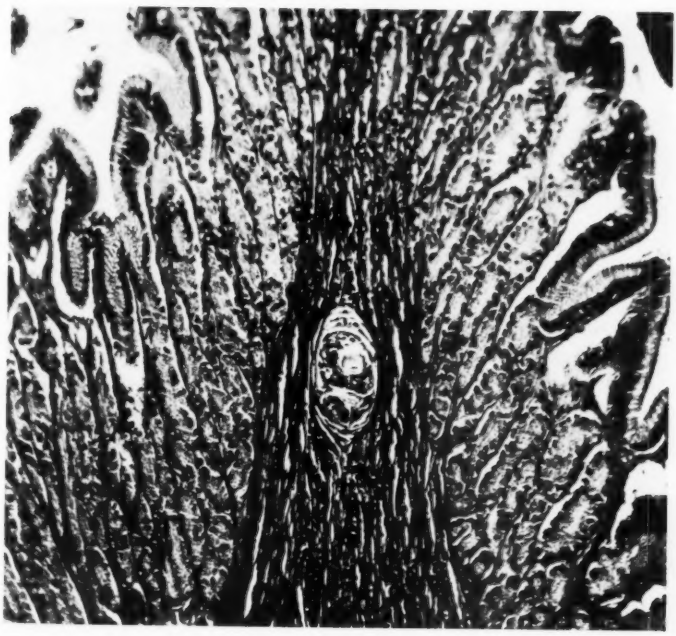

7

Young and Olafson Neurilemomas in Brook Trout

ADENOMA OF THE BOVINE BLADDER *

ROBERT F. LANGHAM, D.V.M., FRANK THORP, JR., D.V.M., and E. T. HALLMAN, D.V.M.
(From the Animal Pathology Section, Michigan Agricultural Experiment Station, East Lansing, Mich.)

Adenoma of the bladder is a rare form of neoplastic disease, and only isolated cases have been published.

Feldman [1] reported a mucoid papillary adenoma associated with calculi in the bladder of a 4-year-old cow. The tumor consisted of a fleshy mass which was firmly attached to the mucosa of the body of the bladder by a rather broad base. Many small, yellowish, cystic foci were present throughout the tumor. Feldman [2] later mentioned another papillary adenoma of the urinary bladder of a bovine. The interior of the bladder was literally filled with hundreds of flattened, string-like prolongations of neoplastic tissue, many of which were approximately 5 cm. long. In Feldman's [2] book the adenoma of the bladder depicted (Fig. 135, p. 284) resembles the microscopic picture exhibited by the two adenomas herein described.

Feldman [2] mentioned Berg [3] and Grips [4] (quoted also by Fölger [5]) as reporting adenoma of the urinary bladder in the bovine. Ewing [6] discussed the isolated reports of adenoma and adenocarcinoma of the bladder in human cases. Mihalovici [7] noted adenomas of the bladder neck upon gross examination (human case). Makar and Urquhart [8] described the gross and microscopic appearances of an adenoma of the bladder in a young man and Paschkis [9] described five cases of adenoma of the bladder in man.

GROSS PATHOLOGY

In studies of bovine pyelonephritis [10] abnormal growths in the bladder were found in two cases.

Case 1820. Guernsey, female, age 12 years. The bladder wall on palpation was thicker than normal. Many firm nodules were detected through the wall. On section the mucosa was markedly congested and showed some hemorrhage. The nodules were pedunculated growths extending from the bladder wall into the lumen (Fig. 1). Except for one area measuring 10 by 10 cm., the mucosa was in irregular folds. The abnormal growths cut with increased resistance and showed an arborescent pattern.

Case 6270. Guernsey, female, age 10 years. The gross appearance of the bladder was similar to that of case 1820 except that there were fewer pedunculated growths.

* Published with the permission of the Director of the Michigan Agricultural Experiment Station as journal article no. 650, new series.
Received for publication, June 7, 1943.

The neoplastic tissues were fixed in Zenker's fluid and stained with hematoxylin and eosin. Gram-Weigert stains for bacteria were also prepared.

Histopathology

Microscopic examination of the nodules in the bladder revealed a neoplasm with the characteristics of an adenoma. The tumors were characterized by a marked proliferation of both epithelial and connective tissue cells. The tumor cells produced a large number of irregular-sized and tortuous gland-like structures (Fig. 2). The epithelium varied from simple to stratified columnar. Many of the gland-like structures disclosed large numbers of goblet cells (Fig. 3). Some parts of the adenoma appeared to have been distended with fluid to form cysts. The epithelium of the gland-like structures near the mucosal surface showed extensive desquamation (Fig. 4).

The stroma appeared to be of a loose mixed type in the more normal areas. In other parts of the neoplasm the stroma exhibited numerous lymphocytes, plasma cells and some mononuclear phagocytes. In a few areas concentric rings of collagenous fibers were found around some of the gland-like structures.

In most areas the transitional epithelium of the bladder mucosa had desquamated or was flattened.

Examination of sections stained by the Gram-Weigert method revealed bacteria (*Corynebacterium renale*) among the desquamated cells scattered along the surface of the bladder mucosa. Bacteria were not observed in the neoplastic tissue.

Summary

A microscopic study of the neoplastic tissue from two bovine bladders revealed adenomatous structures with mucin-producing, columnar epithelium. There was no evidence of infiltrative epithelial growth.

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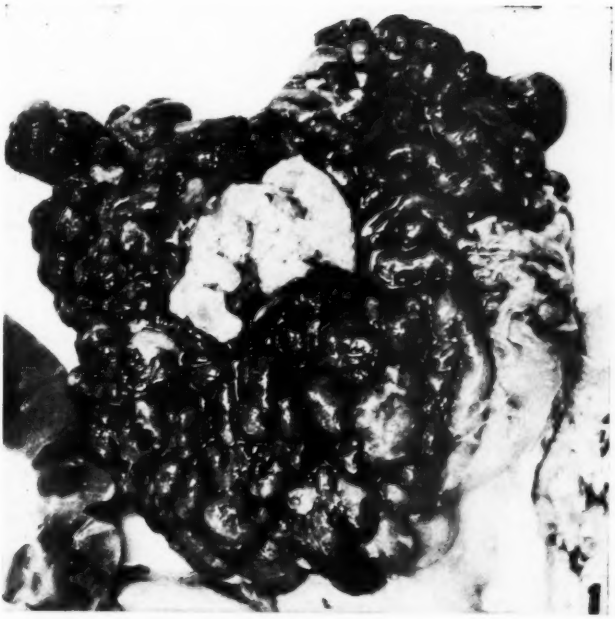
[*Illustrations follow*]

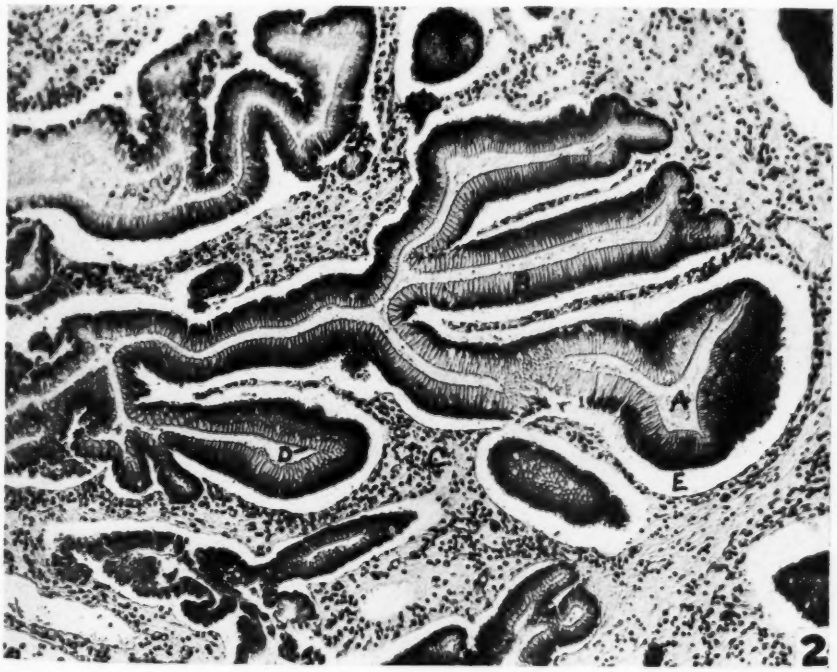
DESCRIPTION OF PLATES

PLATE 82

FIG. 1. Mucosal surface of bladder showing small nodular adenomas. The white material in the center is a purulent and fibrinous exudate. × ½.

FIG. 2. Adenoma of bladder showing (A) gland-like structures of adenoma; (B) pseudostratified columnar epithelium; (C) stroma containing large numbers of lymphocytes and plasma cells; (D) goblet cells; (E) separation of the epithelium from the stroma is an artifact. × 98.



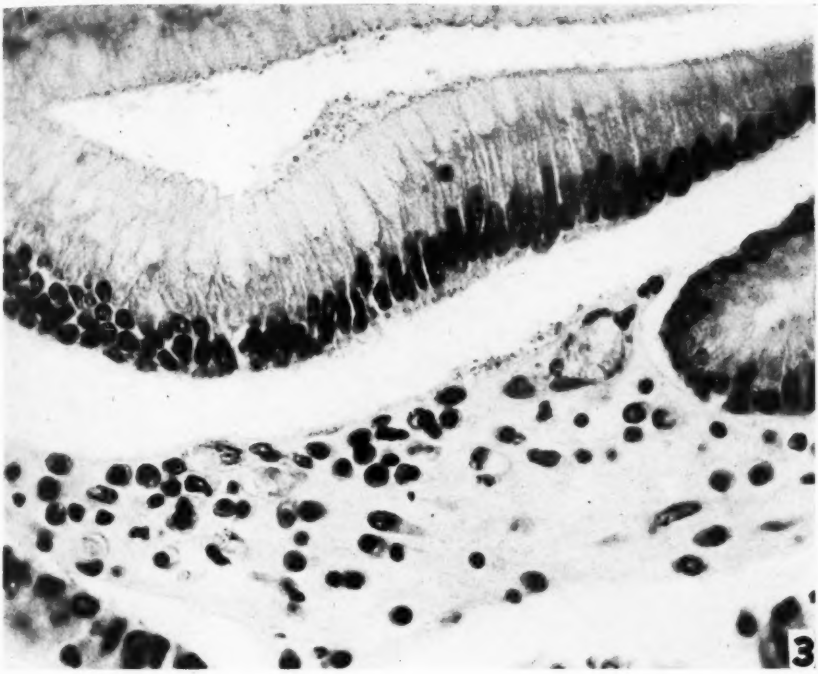


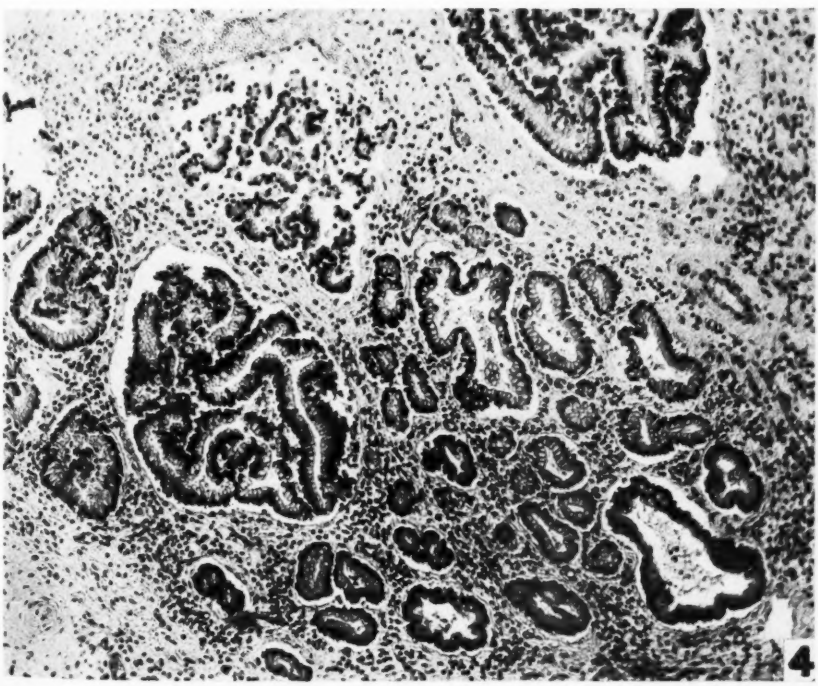
Langham, Thorp and Hallman Adenoma of the Bovine Bladder

PLATE 83

FIG. 3. Higher magnification of Figure 2. × 522.

FIG. 4. Section showing desquamation of the epithelium and the irregularity in size of the gland-like structures. × 98.





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